The evidence that exists in support of biological plausibility of UFP inhalation for effect on male and female reproduction and fertility and pregnancy, birth weight and birth outcomes follows in Figure 9-4. Initial events begin when particles are translocated/solubilized to the lung or the olfactory bulb with the potential for inflammation and oxidative stress. UFP and its soluble components may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of UFP may deposit on the olfactory epithelium. UFP and its soluble components may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4. UFP inhalation by adult male laboratory animals manifests with increased testicular testosterone and its precursor testicular cholesterol (Li et al., 2012). Prenatal exposure of laboratory animals to UFP CAPS results in offspring with decreased kidney weight (Li et al., 2009). The epidemiologic evidence for biological plausibility shows that UFP exposure is associated with low birth weight (Laurent et al., 2014) and preterm birth (Laurent et al., 2016). The biological plausibility for reproductive and developmental outcomes including effects on reproduction and fertility; and pregnancy, birth weight and birth outcomes is emerging. As future studies evaluate the effects of UFP inhalation, more data may become available to elucidate biological plausibility of reproductive and developmental effects.

Inhalation of UFP could lead to effects on male and female reproduction and developmental health effects as well as pregnancy, birth outcomes and birth weight following multiple pathways that are currently sparsely populated. Potential pathways involve, particle translocation/solubility, inflammation and oxidative stress, that may lead to changes in the offspring inducing, altered male reproductive hormone levels, decreased growth and development (e.g., low birth weight), or preterm birth. Evidence from laboratory animals and from epidemiologic studies show that there is potential for growth in the understanding of how the biological plausibility of inhaled UFP affect reproductive and developmental apical events. These limited data provide biological plausibility for epidemiologic results of reproductive and developmental health effects and will be used to inform a causality determination, which is discussed later in the chapter (Section 9.3.4).

9.3.1.2 Male Reproductive Function

The 2009 PM ISA (U.S. EPA, 2009) did not contain studies of UFP in association with male reproductive function. In more recent studies (Table 9-13), UFP exposure has been examined for its effects on male reproductive hormones and sperm production. In these studies, UFP size ranged from 1−100 nm with peak size concentration occurring at 20−30 nm (Li et al., 2009). A couple of studies of DE with adult or prenatal exposures have explored these effects in rodents (Li et al., 2012; Li et al., 2009). Adult male mice were exposed to low dose-DE (LD-DE), high dose-DE (HD-DE), filtered-DE (F-DE) or control clean air for 8 weeks (Li et al., 2012). The HD-DE male mice had significantly higher serum testosterone ($p < 0.05$) than the control or the F-DE; LD-DE showed a nonsignificant trend of

increased testosterone production. Most hormones were refractory to DE exposure (FSH, LH, and progesterone) with 8 weeks of exposure (Li et al., 2012). Epidydimal sperm count and morphology were refractory to PM exposure (Li et al., 2012). Cholesterol is an essential substrate for testosterone production; testicular cholesterol biosynthesis pathways (HMG-CoA reductase, HMG-CoA synthase, LDLR) were significantly upregulated ($p < 0.05$) with HD-DE exposure compared to F-DE and control (Li et al., 2012). Other endpoints essential to testosterone biosynthesis were also significantly upregulated with HD-DE exposure v. control or F-DE exposure (SR-B1, PBR, StAR, P450scc, 3B-HSD, P45017a, 17B-HSD, $p < 0.05$) (Li et al., 2012). In a separate study, the same laboratory also explored prenatal effects of DE on young male offspring, exploring many of the same hormone pathways and looking at male reproductive tract histology (Li et al., 2009). Pregnant dams were exposed to DE, F-DE or control clean air over GD1−19. Immature male offspring were evaluated on PND28. Message levels (mRNA) of FSH receptor and serum concentrations of corticosterone were significantly increased with DE exposure compared to F-DE and control ($p < 0.01$). In these younger mice, other hormone and histology endpoints changed with DE exposure, but they also changed with F-DE exposure compared to control (Li et al., 2009), indicating a gaseous contribution to the DE effect not a PM-specific effect. There were sensitive windows of exposure to UFP PM; exposure of adult males to UFP PM from DE was associated with significantly elevated testosterone but prenatal exposure was not sufficient to induce similar changes in younger male animals. In summary, UFP exposure did not affect rodent sperm count or morphology. Inhalation of UFP in adult animals was associated with changes in concentrations of contributors to the testicular cholesterol biosynthesis pathways including testicular cholesterol, SR-B1, PBR, StAR, P450scc, 3B-HSD, P45017a, and 17B-HSD that likely contributed to the UFP dependent elevated serum testosterone.

9.3.1.3 Female Reproduction and Fertility

No studies on female reproduction and fertility were in the 2009 PM ISA (U.S. EPA, 2009) and no recent studies exist for these health outcomes.

Table 9-13 Key animal toxicological studies UFP and male and female reproduction.

Study	Population N, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
(Li et al., 2009)	Pregnant and lactating F344 rats and their offspring	Pregnant F344 rats were exposed to DEP (148.86 g/m^3, 1.83 × 106 particles/cm^3, 3.40 ppm CO, 1.46 ppm NO_X), filtered-DE (F-DE; 3.10 g/m^3, 2.66 particles/cm^3, 3.30 ppm CO, 1.41 ppm NO_X), or clean air (as a control) from gestation days 1 to 19. UFP size ranged from 1−100 nm with peak size concentration occurring at 20−30 nm.	Male offspring were examined on postnatal Day 28 for endpoints including reproductive organ weight, and hormone concentrations (testosterone, LH, FSH, STAR protein, and 17B-OH dehydrogenase).
(Li et al., 2012)	Adult male C57BL/Jcl mice	Male C57BL/Jcl mice were exposed to clean air, low-dose NR-DE (Low NR-DE), high-dose NR-DE (High NR-DE), or filtered diesel exhaust (F-DE) for 8 weeks at respective PM concentrations of 0.78±0.25, 41.73±0.58, 152.01±1.18, or 0.69±0.36 µg/m^3. UFP size ranged from 1−100 nm with most particles of 20−30 nm in size.	After 8 weeks exposure to DE, F-DE or clean air, isolated testicular interstitial cells from exposed animals were challenged with HCG to understand testicular testosterone production and the role of its precursors (cholesterol, HMG-COA, LDL-R, SR-B1, 17BHSD)

9.3.2 Pregnancy and Birth Outcomes

9.3.2.1 Biological Plausibility

There is a paucity of evidence for biological plausibility of health effects following exposure to UFP due to a dearth of information published in the literature. Thus, a biological plausibility figure was not constructed for this UFP pregnancy and birth outcomes. There have been a limited number of studies of pregnancy and birth outcomes focused on UFP exposure; of these, few examine the same outcome. The studies are reported below.

9.3.2.2 Pregnancy and Birth Outcomes

Limited epidemiologic evidence exists for UFP exposure and pregnancy and birth outcomes. Evidence for effects on birth outcomes includes the results of two, California-based studies using the University of California Davis/CIT_Primary (UCD_*P*) chemical transport model to estimate concentrations. The first, a cohort study of births in Los Angeles county, found increased odds of low birth weight with IQR increases in $PM_{0.1}$ (Laurent et al., 2014). The second, a case-control study of births across the state, found increased odds of preterm birth with increases in $PM_{0.1}$ (Laurent et al., 2016).

Animal toxicology studies routinely measure birth outcomes including birth weight and crown to rump length, measures which have the potential to be affected by UFP PM exposure (Table 9-15). Dams were exposed to control clean air, UFP diesel exhaust (UFP DE), sized 100 nm or less with the majority of the particles of 20−30 nm in size, or F-DE during pregnancy (GD1−19, 5 hours/day) and litter parameters were reported at birth (Li et al., 2013). No markers of maternal endocrine function (dam body weight gain, liver weight, serum maternal LH and corticosterone, corpus luteum 450SSC, 3β-hydroxysteroid dehydrogenase, 17B-estradiol and LH receptor mRNA) were altered with F-DE or DE exposure in pregnant female rats. Both DE and F-DE pups had significantly increased birth weights and significantly decreased crown to rump length at birth versus clean air, indicating that the PM portion of exposure is likely not contributing to the deficit. Also, sex ratio or the ratio of males to females per litter was not altered between treatment groups and neither was anogenital distance, a marker of androgenization. In summary, from this study the UFP PM portion of DE was not responsible for changes in birth weight, crown to rump length, sex ratio, or anogenital distance with prenatal PM exposure.

Table 9-14 Animal toxicological study of pregnancy and birth outcomes.

Study	Population N, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
(Li et al., 2013)	Pregnant female Fischer rats (F344/DuCrlCrli)	Pregnant rats were exposed to DE, F-DE or clean air for the entire pregnancy. Particle size: the average diameter of UFP ranged from 22 to 27 nm. Concentration: DE (148.86 $\mu g/m^3$, 1.83 × 106 particles/cm^3), F-DE (3.10 $\mu g/m^3$, 2.66 particles/cm^3). Inhalation for 5 h/day GD 1 to GD19. UFP size ranged from 1−100 nm with peak size concentration occurring at 20−30 nm.	At birth, maternal outcomes (liver weight, spleen weight, hormone concentrations) were assessed and birth outcomes (birth weight, crown to rump length) were followed in pups.

9.3.3 Developmental Effects

Prenatal or early neonatal exposures have the potential to affect developing organs. Multiple studies characterized in the neurodevelopment section and briefly below show the effects of UFP PM on the nervous system after early life exposure of laboratory rodents to UFP PM, the section that provides the bulk of the new research in the UFP PM Developmental Effects section. These studies find that early life UFP PM exposure to laboratory rodents induces neurobehavioral changes like inattention and depression. Also, brain structures are changed in ways that are similar to the diseases autism or schizophrenia with ventricular enlargement or ventriculomegaly. Also, stress axes like the sympathetic nervous system were differentially activated with UFP PM exposure. These neurological outcomes differ by the sex of the animal tested and by the developmental exposure window (prenatal versus neonatal). Also noted, prenatal UFP PM exposure is associated with decreased kidney size in young male animals; the kidneys of the young male offspring (PND28) prenatally exposed to UFP (DE) were significantly smaller than control clean air exposed animals or F-DE exposed animals ($p < 0.01$) (Li et al., 2009). Dams were exposed to UFP PM DE, F-DE or control clean air 5 hours/day GD1−19. The neuro-developmental studies are characterized below in Section 9.3.3.1 and in Table 9-16.

9.3.3.1 Neurodevelopmental Outcomes

9.3.3.1.1 Neurobehavioral Outcomes, Animal Toxicology

A series of studies evaluated behavioral and neurotoxicological endpoints in adult mice previously exposed to Rochester, NY concentrated ambient ultrafine particles (CAPs) (<100 nm) during the first two weeks of life (Allen et al., 2014b; Allen et al., 2014c; Allen et al., 2014a; Allen et al., 2013). These studies are covered in greater detail in the nervous system section of the ISA (Chapter 8) with brief summaries here. Allen et al. (2013) showed early postnatal CAPs exposure produced mice with preference for immediate with serum corticosterone and some brain region-specific neurotransmitters correlated with measures of impulsivity-linked behavior in male mice. In a second study with similar study design using early life (postnatal) CAPs exposure, Allen et al. (2014c) showed indices of learning/memory were affected by PM. Davis et al. (2013) saw that PM exposure affected internalizing behavior in offspring of dams that were exposed to UFP (prior to conception, mated with unexposed males and then exposed to UFP during gestation). In summary, learning and memory were significantly impaired with UFP exposure, with novel object recognition affected in males (postnatal UFP exposure) and changes in time to approach novel objects affected in females (postnatal UFP exposure). UFP exposure both prenatally and postnatally induced depression like behavior; prenatal exposure's effects were limited to male offspring. UFP exposure did not contribute to anxiety.

9.3.3.1.2 Changes in Brain Structure, Animal Toxicology

Allen et al. (2014a) and Allen et al. (2015) examined changes in the brains of weanling mouse pups exposed postnatally to UFP. Ventriculomegaly was seen in young and adult male, but not female mice. Ventriculomegaly can be associated with increased risk of adverse neurodevelopmental outcomes including schizophrenia ADHD or autism spectrum disorders, some of which tend to have a higher incidence in males. In addition, there was a UFP-dependent decrease in size (PND14, both sexes) and myelination (PND14, males only) of the corpus callosum. Findings of ventriculomegaly, reductions in corpus callosum size, and hypomyelination, especially in males, are consistent with morphologic changes associated with neurodevelopmental disorders such as autism spectrum disorder in humans. There were also sex-specific and region specific alterations in neurotransmitters and hormones (concentration of glutamate, dopamine, norepinephrine, GABA, HVA and corticosterone as well as dopamine turnover (Allen et al., 2014c). Multiday exposure of weaning mice to UFP induced early (astrocyte and microglial) and persistent (microglial) activation, especially in males (Allen et al., 2014a) (Allen et al., 2015).

Table 9-15 Summary of UFP: Developmental outcomes.

Developmental Effects	Summary of Evidence	Cross-link to Study Details	Causality Determination
Neurodevelopment	Toxicological evidence: Early postnatal UFP exposure, Behavioral testing for impulsivity; Early postnatal and adult UFP exposure, measurements of potential brain ventriculomegaly, neurochemical disruption, and glial activation. Sex-dependent measurements; Susceptibility to induction of the Parkinson's disease phenotype (PDP) in adulthood following neonatal CAPS exposure, locomotion activity, and striatal GABA inhibitory function; Measurement of meso-corticolimbic monoamines/glutamate, brain glial activation, and brain histopathology; cerebral cortex primary neuronal cultures; locomotor activity and anxiety-related parameters by open field and elevated plus-maze; depression-like responses by tail-suspension tests.	Section 8.6.6	A Causal relationship is likely to exist for long-term exposure to UFP and nervous system effects
Renal	Toxicological evidence: Kidney development in male offspring, kidney weight. is impacted by $PM_{2.5}$ exposure.	Section 9.3.3	

9.3.4 Summary and Causality Determination

Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between UFP exposure and male and female reproduction and fertility. Causality determinations are made for developmental outcomes in the specific chapters associated with the developmental outcome (i.e., nervous system). This causality determination is consistent with the 2009 PM ISA, which also reported limited evidence for reproductive and developmental effects in association with UFP exposure. The key evidence supporting the causality determination is detailed below using the framework described in Table I of the Preamble to the ISAs (U.S. EPA, 2015)and is presented in Table 9-16. All available evidence examining the relationship between exposure to UFP male and female reproduction and fertility as well as pregnancy and birth outcomes was thoroughly evaluated.

9.3.4.1 Male and Female Reproduction and Fertility

At the time of the 2009 PM ISA (U.S. EPA, 2009), there were not a lot of studies on UFP. The paucity of evidence for UFP in the 2009 PM ISA (U.S. EPA, 2009) remains, however there has been an expansion of studies in neurodevelopment in the laboratory animal toxicology literature. Limited evidence for effects on male reproductive function is provided by the animal toxicology literature which shows increased testosterone, increased testicular cholesterol, and increased activation of biomarkers related to testicular cholesterol biosynthesis with UFP exposure. The evidence for these determinations is contained below in Table 9-16.

Overall, many uncertainties remain when evaluating the evidence for these health endpoints; therefore, **the evidence is inadequate to infer the presence or absence of a causal relationship between UFP exposure and male and female reproduction and fertility.**

Table 9-16 Summary of evidence that is inadequate to infer the presence or absence of a causal relationship between UFP exposure and male and female reproduction and fertility.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	UFP Concentrations Associated with Effects[c]
Reproduction and Fertility: Limited and supportive toxicological evidence of effects on male reproductive endpoints	Adult UFP exposure induced increased testosterone and increased testicular cholesterol, increased activation of biomarkers on testicular cholesterol biosynthesis pathway	(Li et al., 2012)	149 μg/m^3

Table 9-16 (Continued): Summary of evidence that is inadequate to infer the presence or absence of a causal relationship between UFP exposure and male and female reproduction and fertility.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	UFP Concentrations Associated with Effects[c]
Limited evidence for biological plausibility.	Adult UFP impaired testicular T synthesis and biomarkers along the pathway.	(Li et al., 2012)	
Uncertainty regarding exposure measurement error	Chemical transport model to predict UFP concentrations with a 4-km spatial resolution		
Uncertainty regarding epidemiologic evidence from copollutant models to support and independent UFP association	No studies examine potential confounding of UFP associations by copollutants	Section 9.3.1.1	
Uncertainty due to limited biological plausibility from studies of male and female reproduction and fertility; pregnancy and birth outcomes	Dearth of evidence for biological plausibility related to (1) male and female reproduction and fertility.	Sections 9.3.1.1	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm and greater than a nominal diameter of 2.5 μm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{10-2.5}$ concentrations with which the evidence is substantiated.

9.3.4.2 Pregnancy and Birth Outcomes

Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between UFP exposure and pregnancy and birth outcomes. This causality determination is consistent with the 2009 PM ISA, which also reported limited evidence for reproductive and developmental effects in association with UFP exposure. The key evidence supporting the causality determination is detailed below using the framework described in Table I of the Preamble to the ISAs (U.S. EPA, 2015, HERO ID) and is presented in Table 9-17. All available evidence examining the relationship between exposure to UFP and pregnancy and birth outcomes was thoroughly evaluated.

At the time of the 2009 PM ISA (U.S. EPA, 2009), there were not a lot of studies on UFP. The paucity of evidence for UFP in the 2009 PM ISA (U.S. EPA, 2009) remains. Pregnancy and birth

outcomes show positive associations of UFP with preterm birth and low birth weight. There is limited evidence for biological plausibility in support of the reproductive and developmental outcomes. The evidence for these determinations is contained below in Table 9-17.

Overall, many uncertainties remain when evaluating the evidence for these health endpoints; therefore, **the evidence is inadequate to infer the presence or absence of a causal relationship between UFP exposure and pregnancy and birth outcomes.**

Table 9-17 Summary of evidence that is inadequate to infer the presence or absence of a causal relationship between UFP exposure and pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	UFP Concentrations Associated with Effects[c]
Pregnancy and birth outcomes: Limited epidemiologic evidence for associations with pregnancy and birth outcomes	Two studies utilize exposure model for $PM_{0.1}$ to examine associations with birth weight and preterm birth	Section 9.3.2.2 Laurent et al. (2014) Laurent et al. (2016)	1.13 µg/m^3
Uncertainty regarding exposure measurement error	Chemical transport model to predict UFP concentrations with a 4-km spatial resolution		
Uncertainty regarding epidemiologic evidence from copollutant models to support and independent UFP association	No studies examine potential confounding of UFP associations by copollutants	Section 9.3.2	
Uncertainty due to limited biological plausibility from studies pregnancy and birth outcomes	Dearth of evidence for biological plausibility related to pregnancy and birth outcomes.	Section 9.3.2.1	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 µm; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal diameter of 2.5 µm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{10-2.5}$ concentrations with which the evidence is substantiated.

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CHAPTER 10 CANCER

Summary of Causality Determinations for Long-Term Particulate Matter (PM) Exposure and Cancer

This chapter characterizes the scientific evidence that supports causality determinations for long-term PM exposure and cancer. The types of studies evaluated within this chapter are consistent with the overall scope of the ISA as detailed in the Preface (Section 11P.3.1). In assessing the overall evidence, strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Appendix. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015).

Size Fraction	Causality Determination
$PM_{2.5}$	Likely to be Causal
$PM_{10-2.5}$	Suggestive of, but not sufficient to infer
UFP	Inadequate

10.1 Introduction

10.1.1 Evaluation of the Relationship Between Long-term PM Exposure and Cancer

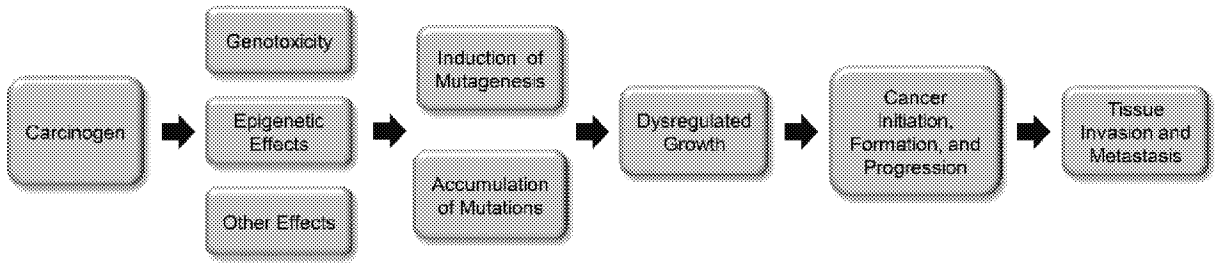
The 2009 Particulate Matter Integrated Science Assessment (2009 PM ISA) evaluated the relationship between long-term PM exposure and cancer, with an emphasis on specific PM size fractions ($PM_{2.5}$, $PM_{10-2.5}$, and UFPs) (U.S. EPA, 2009), with most studies focused on $PM_{2.5}$ exposure. This body of evidence was supported by decades of research on whole PM exposures (i.e., no defined size fraction), including diesel exhaust, gasoline exhaust, and wood smoke.

Since completion of the 2009 PM ISA, the International Agency for Research on Cancer (IARC) classified outdoor air pollution, including PM, as a Group 1 carcinogen (carcinogenic to humans) (IARC, 2016). IARC conducted a weight-of-evidence assessment for hazard identification that involved evaluating epidemiologic, animal toxicological, and mechanistic studies associated with outdoor air pollution. Studies evaluated in the IARC assessment consisted of those that examined inhalation as well as other routes of exposure, PM concentrations higher than 1−2 orders of magnitude above ambient, and individual PM components and specific PM size fractions. The conclusion of the IARC assessment was based primarily on epidemiology studies of ambient $PM_{2.5}$ exposures and lung cancer incidence and

mortality, on inhalation studies of promotion-initiation in mice exposed to ambient air PM_{10}, and on evidence from mechanistic studies using PM of various size fractions. In contrast, this ISA is tasked with evaluating only inhalation exposures of specific PM size fractions at relevant ambient concentrations (i.e., up to one to two orders of magnitude above ambient). The evaluation of the relationship between long-term exposure to $PM_{2.5}$, as well as other PM size fractions, and cancer is guided by the overall scope of the ISA as detailed in the Particulate Matter Integrated Review Plan (U.S. EPA, 2016) and summarized briefly in the Preface (Section P.3.1).

10.1.2 Carcinogens and the Development of Cancer

Development of cancer is a complex, multistep disease process (Figure 10-1). Evidence collected over decades of scientific research suggests that dysregulation of cellular pathways controlling cell growth, survival, and genetic stability results in aberrant, unregulated cell division and is central to disease initiation and progression. The most widely accepted pathway to unregulated growth is accumulation of mutations in critical genes. However, more recently, epigenetic mechanisms, such as gene silencing through promotor methylation, or receptor-mediated cell proliferation have been proposed to be important to disease development (Smith et al., 2016).



Note: This scheme depicts important steps in the development of cancer and is adapted from Goodson et al. (2015) and Smart et al. (2008).

Figure 10-1 Key steps in the development of cancer.

Hanahan and Weinberg (2000) and Hanahan and Weinberg (2011) have proposed several hallmarks of cancer that describe the phenotype of cancer cells and developed tumors. These hallmarks organize the dysregulated pathways identified in cancer cells in terms of biological properties that are acquired during tumor development in humans (Hanahan and Weinberg, 2011). They include sustained proliferative signaling, evasion of growth suppressors, resistance of cell death, enabling of replicative immortality, induction of angiogenesis, activation of invasion and metastasis, reprogramming of energy

metabolism, and evasion of immune destruction. Few studies of exposure to PM size fractions have specifically examined dysregulated pathways associated with cancer cells and developed tumors. However, as described below, some studies of exposure to PM size fractions demonstrate perturbation of pathways related to the hallmarks of cancer, such as methylation of a tumor suppressor gene, which is relevant to evasion of growth suppressors.

Smith et al. (2016) has proposed ten characteristics of carcinogens as important to the etiology and progression of cancer. These characteristics are related to the mechanisms through which it is currently thought carcinogenic agents act. These characteristics include the ability to (1) be electrophilic either directly or after metabolic activation, (2) be genotoxic, (3) alter DNA repair or cause genomic instability, (4) induce epigenetic alterations, (5) induce oxidative stress, (6) induce chronic inflammation, (7) be immunosuppressive, (8) modulate receptor-mediated effects, (9) cause immortalization, and (10) alter cell proliferation, cell death, or nutrient supply. Numerous studies published prior to the 2009 PM ISA showed that PM of various size fractions exhibit many of these characteristics, especially the first six (IARC, 2016). Studies published since the 2009 PM ISA provide evidence that the PM size fractions of interest in this ISA, (i.e., $PM_{2.5}$, $PM_{10-2.5}$, and UFP) exhibit several of the key characteristics of carcinogens. New findings describe the capability of these PM size fractions to induce oxidative stress and to damage DNA, which can be processed by the cell into gene and chromosomal mutations. Furthermore, studies link PM size fractions to the expression of genes that are relevant to metabolic activation or biotransformation and to epigenetic alterations.

In addition to consideration of the hallmarks of cancer (Hanahan and Weinberg, 2000); (Hanahan and Weinberg, 2011) and the characteristics of carcinogens (Smith et al., 2016), studies examining the effects of exposure to PM size fractions provide information on other cancer-related biomarkers. Some studies detail the presence of mutagenic compounds in PM size fractions collected from ambient air, while others measure the formation of DNA adducts and carcinogenic potential.

10.2 $PM_{2.5}$ Exposure and Cancer

The 2009 PM ISA concluded that the overall body of evidence was "suggestive of a causal relationship between relevant $PM_{2.5}$ exposures and cancer" (U.S. EPA, 2009).[76] This conclusion was based primarily on positive associations observed in epidemiologic studies of lung cancer mortality. Epidemiologic studies evaluating $PM_{2.5}$ and lung cancer incidence or cancers of other organs and systems generally did not show evidence of an association. Toxicological studies did not focus on exposures to specific PM size fractions, but rather investigated the effects of exposures to total ambient PM, or other source-based PM such as wood smoke. Collectively, results of in vitro studies were consistent with the

[76] As detailed in the Preface, risk estimates are for a 5 $\mu g/m^3$ increase in annual $PM_{2.5}$ concentrations unless otherwise noted.

larger body of evidence demonstrating that ambient PM and PM from specific combustion sources are mutagenic and genotoxic. However, animal inhalation studies found no evidence of tumor formation in response to chronic exposures, except for one study demonstrating enhanced formation of urethane-induced tumors. In addition, a small number of studies provided preliminary evidence that PM exposure can lead to changes in methylation of DNA, which may also contribute to biological events related to cancer.

Recent studies expand upon the evidence for long-term $PM_{2.5}$ exposure and cancer detailed in the 2009 PM ISA. Although previous studies tended to focus more broadly on PM exposures, recent studies address a number of uncertainties and limitations with respect to the role of $PM_{2.5}$ exposure in the development of cancer. Evidence from experimental and epidemiologic studies demonstrate that $PM_{2.5}$ exposure can lead to a range of effects indicative of mutagenicity, genotoxicity, and carcinogenicity, as well as epigenetic effects. These cellular and molecular changes are supported by epidemiologic evidence demonstrating consistent positive associations between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence.

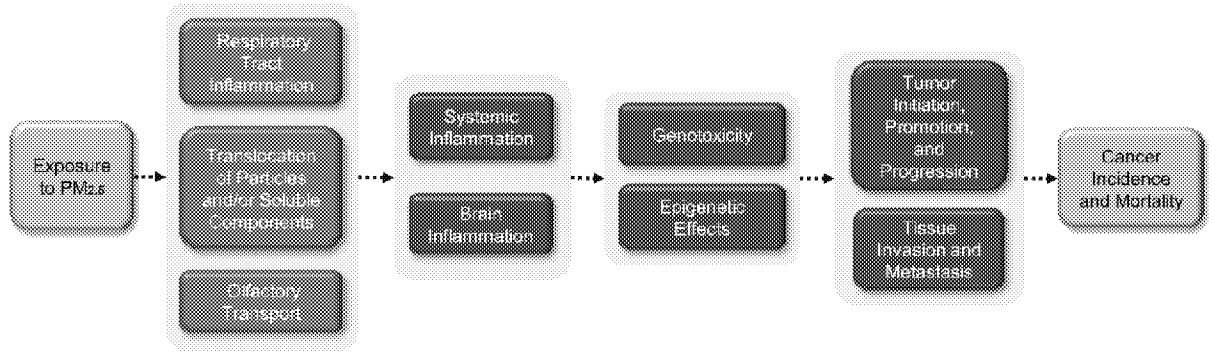
The following sections evaluate studies published since completion of the 2009 PM ISA. Although the ISA is tasked with reviewing new evidence describing the mutagenicity, genotoxicity, and carcinogenicity for each PM size fraction, it is recognized that there exists a large body of historical evidence demonstrating these effects resulting from exposure to total PM. Throughout this section recent studies are evaluated in the context of this larger collective body of evidence.

10.2.1 Biological Plausibility

This section describes biological pathways that potentially underlie the development of cancer resulting from exposure to $PM_{2.5}$. Figure 10-2 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to $PM_{2.5}$ may lead to the development of cancer contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 10.2.

Once $PM_{2.5}$ deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). $PM_{2.5}$ and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate reactive oxygen species (ROS) and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see CHAPTER 4). Immune system responses due to the presence of particles in the interstitial space may contribute to chronic health effects. Inflammatory

mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (see Chapter 6). Soluble components of $PM_{2.5}$ and poorly soluble particles that are part of the $PM_{2.5}$ fraction and smaller than approximately 200 nm may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of $PM_{2.5}$ may deposit on the olfactory epithelium. Soluble components of $PM_{2.5}$ and poorly soluble particles that are part of the $PM_{2.5}$ fraction and smaller than approximately 200 nm may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4. The potential contribution of olfactory transport to brain inflammation or to upregulation of gene expression in the brain is discussed in Chapter 8.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 10-2 Potential biological pathways for the development of cancer following exposure to $PM_{2.5}$.

Evidence is accumulating that exposure to $PM_{2.5}$ may lead to carcinogenesis by two pathways. The first pathway involves genotoxicity, where electrophilic compounds induce DNA damage, such as DNA strand breaks or DNA adducts (where a compound is bound covalently to DNA), and such damage is then processed by the cell to result in a change in DNA sequence—i.e., a mutation. The second pathway involves epigenetic effects that alter gene expression, further altering cell growth, regulation, and other processes. Carcinogenesis is essentially dysregulated growth; one or the other or a combination of both pathways above can lead to cancer. A general scheme for cancer induction involves initiation, promotion, and progression, leading eventually to tissue invasion and metastasis. Although most of

epidemiologic evidence links $PM_{2.5}$ exposure to lung cancer, a plausible link to other kinds of cancer may exist. Evidence for these pathways and cancer-related biomarkers is described below. A discussion of the hallmarks of cancer (Hanahan and Weinberg, 2000); (Hanahan and Weinberg, 2011) and the characteristics of carcinogens (Smith et al., 2016), as they relate to $PM_{2.5}$, follows.

Genotoxicity

Genotoxicity is a term that refers to DNA damage, mutations, or both (Shaughnessy and DeMarini, 2009). DNA damage consists of alterations to DNA such as a DNA strand break (breakage of the phosphodiester bonds) or a DNA adduct (the covalent binding of a chemical to DNA). The DNA damage itself generally does not alter the sequence or number of the four bases/nucleotides in DNA, whose order form the basis of the genetic code. DNA damage can be caused by spontaneous errors of nucleic acid metabolism or by endogenous or exogenous mutagens. In contrast, mutations are changes in DNA sequence (i.e., in the order or number of the bases/nucleotides), and they occur when the cell processes DNA damage incorrectly, such as by failing to repair the damage or by trying to perform DNA replication past the unrepaired damage. Thus, mutagenesis is a cellular process, usually involving DNA replication and DNA repair. There are three classes of mutations: gene, chromosomal, and genomic. Mutations within a single gene are called gene or point mutations, such as base substitutions. Mutations involving more than one gene are called chromosomal mutations, such as chromosomal aberrations involving multigenetic deletions, inversions, duplications, or translocations. The gain or loss of a whole chromosome (aneuploidy) is an example of genomic mutation. As detailed below, $PM_{2.5}$ exposure is associated with mutagenicity, DNA adducts and other DNA damage, oxidative stress, biotransformation, and chromosomal (or cytogenetic) effects.

Mutations are considered biomarkers of early biological effect (Demetriou et al., 2012). The Ames *Salmonella*/mammalian-microsome mutagenicity assay is a bacterial assay and the most widely used assay of any kind for detecting the mutagenic activity of an agent (Claxton et al., 2010). In the absence of metabolic activation, it detects agents that are called direct-acting mutagens; in the presence of metabolic activation, it detects agents that are indirect-acting mutagens, i.e., those requiring metabolism to electrophilic forms. The somatic mutation theory of cancer is the most widely accepted theory of cancer etiology, and it postulates that cancer occurs at a minimum from the accumulation of mutations in critical genes. The presence of mutagens within PM and the mutagenicity of organic extracts of PM provide biological plausibility for observations made in epidemiologic studies of cancer incidence. Although the Ames assay has several technical limitations and is criticized due to its use of bacteria as a model species, more than four decades of published results evaluating 10,000 compounds have clearly demonstrated the validity of this assay for evaluating the mutagenicity of PM collected from ambient air (Claxton et al., 2010; U.S. EPA, 2009). New studies published since the 2009 PM ISA provide evidence to support mutagenicity resulting from $PM_{2.5}$ exposure (Section 10.2.2.1).

DNA adducts are a type of DNA damage and serve as a biological marker of exposure (Demetriou et al., 2012). They form via a covalent bond between DNA and a carcinogen or a metabolite of a carcinogen. Repair proteins may remove DNA adducts. However, persistent adducts may result in mutations when the DNA polymerase tries to replicate past the adduct, resulting in nucleotide (base) substitutions, deletions, duplications, and chromosome rearrangements. An in vitro toxicological study described in the 2009 PM ISA provides evidence for the formation of DNA adducts following exposure to $PM_{2.5}$ (De Kok et al., 2005). In this study, rat liver S9 metabolism was found to increase DNA reactivity (i.e., the induction of DNA adducts). Supporting evidence is provided by recent epidemiologic studies showing benzo[a]pyrene (B[a]P) -like DNA adducts in association with $PM_{2.5}$ exposure (Li et al., 2014; Rossner et al., 2013b). Other types of DNA damage involve the formation of oxidized bases or nucleotides, as well as the induction of single- or double-strand breaks, all of which can be determined by the comet assay (Demarini, 2013). Evidence for such DNA damage following $PM_{2.5}$ exposure is provided by several in vitro studies using the comet assay and by a study measuring phosphorylated H2AX, which measures double-strand breaks (Section 10.2.2.2). A single epidemiologic study provides supportive evidence for DNA damage, as assessed by the comet assay, in association with $PM_{2.5}$ concentrations (Chu et al., 2015).

Some of the studies examining DNA damage identified oxidized bases, suggesting a role for oxidative stress in the development of the DNA lesions (Section 10.2.2.2). These oxidized DNA nucleobases are considered a biomarker of exposure (Demetriou et al., 2012). Exposure to PM can result in oxidative stress either through the direct generation of ROS, or indirectly through the induction of inflammation. Treatment with an antioxidant blocked strand breaks due to $PM_{2.5}$ exposure (Oh et al., 2011). Other in vitro studies showed that exposure to $PM_{2.5}$ increased the production of reactive oxygen species (ROS) in vitro. The in vitro results are supported by both animal toxicological and controlled human exposure studies. An inhalation study involving $PM_{2.5}$ in male mice found oxidized DNA bases in lung tissue (Soberanes et al., 2012). A study in human subjects found increased lipid peroxidation products in urine (Liu et al., 2015). The presence of oxidative stress-mediated DNA lesions, including adducts, can lead to the introduction of fixed mutations into the genome after incorrect repair of the damaged base or replication past the base by low fidelity DNA polymerases. The potential for oxidative stress to result in mutagenesis is underscored by the DNA repair mechanisms that have evolved to protect the genome from mutagenesis caused by these lesions.

Some components of PM, especially organic compounds, may undergo metabolism in a variety of cell types, resulting in electrophilic compounds that may bind to DNA, RNA, or proteins. Evidence that genes participating in polycyclic aromatic hydrocarbon (PAH) biotransformation are upregulated as a result of $PM_{2.5}$ exposure is provided by in vitro studies Borgie et al. (2015b) and Gualtieri et al. (2011). Biotransformation via Cyp1A1 may result in the production of PAH metabolites capable of reacting with DNA to form bulky DNA adducts. As in the case of oxidative-stress mediated DNA adducts, when DNA repair of bulky adducts is absent or ineffective, mutational events may occur.

Cytogenetic effects, such as micronuclei formation and chromosomal aberrations, are also biomarkers of genotoxicity (Demarini, 2013). Micronuclei are small nuclei formed either by chromosomal breakage or aneuploidy, which is the addition or deletion of a whole chromosome (Demetriou et al., 2012). $PM_{2.5}$ exposure increased micronuclei formation in vitro (Lemos et al., 2016; Oh et al., 2011). This effect was blocked by an antioxidant, suggesting that oxidative stress may play a role (Oh et al., 2011). The formation of micronuclei correlated with the amount of DNA damage detected by the comet assay in the same study. Epidemiologic studies provide supporting evidence of chromosomal aberrations in association with $PM_{2.5}$ exposure (Rossner et al., 2013a; Rossner et al., 2011).

Epigenetic Effects

Epigenetic mechanisms regulate the transcription of genes without altering the nucleotide sequence of DNA. Three sets of epigenetic effects were examined in studies of $PM_{2.5}$: methylation of tumor suppressor genes, global DNA methylation, and alteration in noncoding miRNA. Changes in DNA methylation patterns can affect gene expression and genomic instability (Demetriou et al., 2012). They are considered a biomarker of early exposure. In general, transcription repression is associated with DNA methylation in promoter regions of genes. Inhalation exposure to $PM_{2.5}$ increased methylation of the p16 promotor in the lung (Soberanes et al., 2012). The p16 protein is a tumor suppressor, suggesting an epigenetic mechanism for dysregulated growth. Methylation of repetitive elements, a surrogate of global DNA methylation, was correlated with $PM_{2.5}$ concentrations in blood and lung tissue of Wistar rats (Ding et al., 2016). Global DNA methylation is a measure of genomic instability which can contribute to the accumulation of mutations in critical genes involved in the development of cancer. In general, hypomethylation is associated with genomic instability. In an in vitro study, methylation of repetitive elements and methyltransferase gene expression were decreased due to $PM_{2.5}$ exposure (Miousse et al., 2015). Support for a relationship between $PM_{2.5}$ exposure and global DNA methylation is provided by several epidemiologic studies (Section 10.2.3). Alteration in a third type of epigenetic effect, specific noncoding miRNA, was also found as a result of $PM_{2.5}$ exposure (Borgie et al., 2015b). These effects may contribute to the accumulation of mutations or dysregulated growth.

Carcinogenic Potential

None of the toxicological studies involving $PM_{2.5}$ exposure provides direct evidence of carcinogenesis. However, an animal inhalation study found that $PM_{2.5}$ exposure led to tumor promotion in a model of urethane-induced tumor initiation (Cangerana Pereira et al., 2011). Furthermore, exposure to $PM_{2.5}$ in vitro increased cell invasion, a measure of metastatic potential, which correlated with PAH content (Yue et al., 2015). This effect was blocked by treatment with an antioxidant, suggesting a role for oxidative stress. Epidemiologic studies provide initial evidence that exposure to long-term $PM_{2.5}$ concentrations may contribute to reduced cancer survival (Section 10.2.5.3). This could involve an enhancement of tumor progression or metastasis/tissue invasion or some other mechanism.

Characteristics of Carcinogens and Hallmarks of Cancer

$PM_{2.5}$, as described in the studies evaluated in this chapter, exhibits several characteristics of carcinogens (Smith et al., 2016). Exposure to $PM_{2.5}$ results in genotoxic effects, epigenetic alterations, and oxidative stress. In addition, exposure to $PM_{2.5}$ induces expression of genes involved in PAH biotransformation, indicating that $PM_{2.5}$ contains electrophilic species. Additional studies provide evidence that $PM_{2.5}$ exposure may lead to perturbations of pathways related to the hallmarks of cancer (Hanahan and Weinberg, 2000); (Hanahan and Weinberg, 2011). Findings of enhanced tumor formation may indicate the sustaining of proliferative signaling; increased cell invasion may indicate the activating of invasion and metastasis; methylation of a tumor suppression gene may indicate the evading of growth suppressors; and increased telomerase activity may indicate the enabling of replicative immortality.

Summary of Biological Plausibility

As described here, there are two proposed pathways by which exposure to $PM_{2.5}$ could lead to the development of cancer. The first pathway involves genotoxicity, including DNA damage that could lead to mutational events, such as gene mutation and cytogenetic effects. The second pathway involves epigenetic effects, including methylation of a tumor suppressor gene. Although experimental studies in animals and humans contribute most of the evidence of upstream events, epidemiologic studies report associations between exposure to $PM_{2.5}$ and DNA damage (including DNA adducts), chromosomal mutation (chromosomal aberrations), and epigenetic changes (altered global DNA methylation). Evidence of tumor promotion, a measure of carcinogenic potential, was found in an animal toxicological study. Together, these proposed pathways provide biological plausibility for the epidemiologic results of lung cancer incidence and mortality and will be used to inform a causality determination, which is discussed later in the chapter (Section 10.2.5).

10.2.2 Genotoxicity

In the 2009 PM ISA, there were many toxicological studies that examined mutagenicity, DNA damage, and other endpoints related to genotoxicity. The presence of mutagens in PM extracts collected from ambient air was first demonstrated by Pitts et al. (1975). In agreement with that work and many similar subsequent findings published over the past 40 years, results from studies evaluated in the 2009 PM ISA confirmed that PM and/or PM extracts collected from both ambient air and multiple combustion sources can induce DNA mutations in various strains of *Salmonella* developed by Bruce Ames and others. PM exposure in other in vitro assay systems resulted in changes in molecular and cellular markers that have been associated with genotoxicity. In addition, an in vivo study by Sato et al. (2003) reported increased DNA adducts in lung, liver, and nasal mucosal tissues after inhalation exposure to urban roadside air. Because this study evaluated effects of exposure to a mixture of PM and gases, it does not inform the current ISA, which identifies the hazard for effects after exposures to only the PM component

of complex mixtures. Furthermore, a small number of epidemiologic studies evaluated in the 2009 PM ISA examined molecular and cellular markers that have often been linked with genotoxicity. Many of these studies focused only on PM_{10} exposures or individual components of PM. As a result, these epidemiologic studies did not thoroughly examine the relationship between $PM_{2.5}$ exposure and genotoxicity.

As noted in the 2009 PM ISA, there was a paucity of available studies that investigated the effects of exposures to specific PM size fractions. There were no new studies that evaluated in vivo effects of exposures to $PM_{2.5}$ present in ambient air. Although new in vitro studies were reviewed that confirmed previous reports demonstrating induction of mutagenesis, DNA strand breaks, micronuclei, and oxidative stress after $PM_{2.5}$ and/or $PM_{2.5}$ extract exposures, the relationships between observations from in vitro assays and in vivo endpoints and complex biological disease processes such as carcinogenesis remained uncertain. Moreover, the diversity of in vitro assay protocols and measured endpoints limited the ability to draw more than general conclusions regarding the carcinogenic potential of $PM_{2.5}$.

Since the 2009 PM ISA, new studies continue to investigate mutagenicity, genotoxicity, and carcinogenicity of PM, including many studies that, as in the past, evaluate the effects of total particulate matter (TPM), PM_{10}, and total PM collected from specific combustion sources including diesel and gasoline exhaust and woodsmoke. In addition, recent studies also investigate cancer-related effects following inhalation of $PM_{2.5}$ CAPs, ambient air, and emissions from specific combustion sources. The findings from these studies are supportive of findings from previous studies. However, as discussed in the Preface, the focus of the PM ISA is on the evaluation of the health effects due to exposures to specific PM size fractions (i.e., $PM_{2.5}$, $PM_{10-2.5}$, and UFPs). As a result, in the evaluation of long-term $PM_{2.5}$ exposure and cancer, in the assessment of the experimental evidence for mutagenicity, genotoxicity, and other endpoints associated with carcinogenesis and cancer, the focus is on exposures to $PM_{2.5}$.

10.2.2.1 Mutagenicity

Evidence for mutagenicity is provided by toxicological studies. The Ames *Salmonella*/mammalian-microsome mutagenicity assay has been used for more than 40 years to identify the presence of chemical mutagens (Claxton et al., 2010; Ames, 1971). Developed to screen single chemicals for their potential to induce mutagenesis, the assay was first extended to investigate the mutagenicity of extracted organic material (EOM) from PM collected from air in Los Angeles (Pitts et al., 1975). The *Salmonella* test provided a simple, fast, and inexpensive method for detecting the presence of mutagens within the complex mixture of chemical species that can be present in ambient air.

Assay results over the past 40 years have provided meaningful information regarding the mutagenicity of airborne compounds. The *Salmonella* test, however, is not without technical limitations. For example, it is difficult to draw detailed conclusions based upon direct comparisons between study results because of assay sensitivity to differences in methods. Many studies examine only the organic

matter adsorbed onto collected particles and extraction protocols including solvent and extraction method selection have been shown to affect the amount and class of compounds recovered (Claxton et al., 2004). In addition, several strains of *Salmonella* and variations in assay protocols have been developed. One advantage of the assay is that various strains selectively respond to specific chemical classes, such as nitroarenes, PAHs, or aromatic amines, providing the ability to infer some of the chemical classes responsible for the mutagenicity. However, differences in strains and protocols can modify the reproducibility of results and/or the sensitivity of the assay to certain classes of mutagens (Claxton et al., 2004; Gatehouse et al., 1994). Moreover, studies have revealed that mutagenicity fluctuates seasonally (Claxton et al., 2004). Together, these factors can affect the number of revertant colonies observed and thus limit direct comparisons between disparate studies.

Analyses using various data bases have been performed to see how well the *Salmonella* mutagenicity assay predicts rodent carcinogenicity. The values that have been calculated for both the sensitivity (the percentage of known carcinogens to elicit a positive response in *Salmonella*) and specificity (the percentage of known noncarcinogens to elicit a negative response in *Salmonella*) are 45–80 and 67–100% for sensitivity and specificity, respectively (Kirkland et al., 2005; Zeiger, 1998; Zeiger et al., 1990; Tennant et al., 1987; Kier et al., 1986). Thus, agents that are not carcinogenic in rodents can also be mutagenic in the assay, and some chemical classes of rodent carcinogens are not mutagenic in the *Salmonella* assay (Zeiger et al., 1990). Considering also that PM is a heterogeneous and dynamic mixture with many unknown chemical species, *Salmonella* assay results are accordingly accompanied by uncertainty.

As discussed above, most studies of PM with the *Salmonella* mutagenicity assay evaluated only the EOM adsorbed onto particles. Because extraction results in an enriched preparation of organic compounds, the concentration applied in the assay may not reflect the administered dose delivered to the lung via inhalation of ambient air, nor accurately represent the mixture present on PM as species such as metals and volatile organic compounds (VOCs) will not be responsive to organic extraction. Further, the bioavailability of extracted compounds may not be comparable to the bioavailability of those adsorbed onto particles.

As with many bioassay, the *Salmonella* strains used in the Ames assay have been engineered to improve their ability to detect mutagens. Thus, there is a mutation in a gene coding for a component of the cell wall that makes the cells more permeable to large molecules. This permits PM components such as PAHs to enter the cell and get to the DNA. Likewise, there are various DNA repair deficiencies, such as the elimination of nucleotide excision repair or the addition of error-prone DNA repair, that also enhance the sensitivity of the strains to mutagens. Several different mutations in the histidine genes are present in the strains, permitting the detection of all six types of base substitutions, a 2-base frameshift mutation, as well as some small deletions. One of the most important developments that has made the strains especially useful for complex mixtures is the development of strains with various metabolic capabilities, permitting the inference of specific chemical classes in a complex mixture as being

responsible for some of the mutagenicity of that mixture. Thus, some strains express excess nitroreductase, which activates nitroarenes, and others express acetyltransferase, which can help activate aromatic amines.

Although many new studies using the *Salmonella*/mammalian-microsome assays have been published, only a fraction evaluated the mutagenic activity of $PM_{2.5}$. Of these, all were conducted outside of the U.S. in Brazil, Japan, India, and Italy. In general, the findings support previously published results that organic extracts from collected PM (various size fractions) contain compounds capable of inducing mutagenesis in the *Salmonella* assay. Specifically, results from these studies demonstrate that organic extracts of $PM_{2.5}$ collected from diverse sampling locations exhibit mutagenic activity. The induction of mutations in both the absence and presence of mammalian S9 fractions indicate the presence of compounds that are capable of interacting with DNA without biotransformation as well as those that require metabolic activation to generate ultimate carcinogens (Lemos et al., 2016; Traversi et al., 2014; de Rainho et al., 2013; Rainho et al., 2013; Lemos et al., 2012; Singla et al., 2012; Traversi et al., 2011; Kawanaka et al., 2008; Traversi et al., 2008). In addition to these general findings, several studies also identified the presence of certain compound classes, seasonal variation in mutagenic activity, and the tendency for $PM_{2.5}$ to elicit a greater increase in mutagenicity compared to PM_{10} (Lemos et al., 2016; Traversi et al., 2014; de Rainho et al., 2013; Rainho et al., 2013; Lemos et al., 2012; Singla et al., 2012; Traversi et al., 2011; Kawanaka et al., 2008; Traversi et al., 2008).

As has been documented by past studies, use of plasmid-modified strains sensitive to nitro-PAH species in new studies confirmed the presence of those compounds in airborne particulate matter from sites in Brazil and Italy. Sites included low traffic areas identified as urban background or residential locations (Lemos et al., 2016; de Rainho et al., 2013; Rainho et al., 2013; Lemos et al., 2012; Traversi et al., 2011). For example, Traversi et al. (2011) used three isogenic strains with varying nitroreductase activity to qualitatively demonstrate the contribution of nitroaromatic compounds to the overall mutagenicity observed. $PM_{2.5}$ was most mutagenic in strains with elevated nitroreductase activity, suggesting the presence of nitroaromatic compounds in the extracts evaluated. The knowledge that these compounds are present in PM emissions and that they can induce mutagenesis in the *Salmonella* assay is well established (NTP, 2014; Claxton et al., 2004; Purohit and Basu, 2000; Rosenkranz and Mermelstein, 1983).

Kawanaka et al. (2008) investigated the mutagenicity of EOM from $PM_{2.5}$ collected roadside in Saitama City, Japan. Using a cascade impactor, 12 fractions of varying aerodynamic diameters were collected including fine fractions (<0.12, 0.12–0.20, 0.20–0.30, 0.30–0.50, 0.70–1.2, 1.2–2.1, 2.1–3.5, 3.5–5.2, 5.2–7.8, 7.8–11, >11 μm). The authors used the *Salmonella* assay to determine the mutagenic potency of each fraction and GC/NCI/MS/MS to determine the mass contribution of select nitroaromatic compounds to the total PM mass collected. They used known quantities of those compounds to estimate the contribution of those species to total mutagenicity. Using this approach, the authors reported that the quantity of nitro-PAHs per unit mass in the ultrafine fraction (<0.12) was greater than in that of $PM_{2.5}$ or

$PM_{10-2.5}$. In addition, the authors determined that mutagenicity per unit mass of $PM_{2.5}$ was less than that of UFP in both strains. Moreover, of the six nitroaromatic compounds evaluated, the contribution to mutagenic activity calculated was greatest for 1,8-dinitropyrene in all three fractions of PM extracts evaluated. Due to biological variability of the *Salmonella* assay as well as incomplete details regarding the statistical analysis of the data collected, it is difficult to calculate definitive values for these contributions.

Several studies evaluated seasonal variation in mutagenesis using the *Salmonella* assay (Lemos et al., 2016; Traversi et al., 2011; Traversi et al., 2008). Each observed greater mutagenic activity in extracts from PM collected during the autumn and winter seasons compared to that from PM collected during the spring and summer seasons. These findings agree with previous studies that have also demonstrated the inverse correlation between temperature and mutagenic activity (IARC, 2016; Claxton et al., 2004). Singla et al. (2012) also compared seasonal variation in mutagenic activity. Although the authors did not provide a statistical analysis of the variation in values, they did report a consistent trend in which the mutagenicity of extracts from PM collected during the winter season was greater than the mutagenicity of those from PM collected during the monsoon season. In this study, they suggested that this divergence may be due not only to the increase in temperature, but also to the increase in rainfall.

Singla et al. (2012) and Traversi et al. (2011) analyzed the mutagenic activity of $PM_{2.5}$ and PM_{10} collected during the same timeframes. In experiments using the frameshift strain TA98 without the addition of S9, which especially detects nitroarenes (Singla et al., 2012), the authors reported that the organic extracts collected from $PM_{2.5}$ had higher mutagenic potencies that those from PM_{10}. However, this same effect was not observed in experiments using TA100, which detects primarily PAHs. Likewise in the study by Traversi et al. (2011), the mutagenic potency of the organics extracted from $PM_{2.5}$ was 6.5-fold greater than that from PM_{10} in strain TA98. Further, the authors reported greater mutagenicity for the organic extracts from $PM_{2.5}$ collected in the winter season compared to that from PM_{10} when using the nitro-PAH sensitive YG1021 strain (5.75-fold increase). A third study carried out a similar analysis. Lemos et al. (2012) compared the mutagenic activity of organic extracts from $PM_{2.5}$ and total suspended particles (TSP). The authors reported that the mutagenic potencies of the organic extracts from $PM_{2.5}$ were generally greater than those from TSP; however, a statistical analysis was not provided. Lemos et al. (2012) showed that an aqueous extract generated sequentially after the organic extraction was not mutagenic, showing that all the measured mutagenic activity was in the organic extract.

In summary, while the Ames *Salmonella*/mammalian-microsome mutagenicity assay has several technical limitations and is criticized due to its use of bacteria as a model species, four decades of published results from this assay have clearly demonstrated the presence of mutagenic agents in PM of various size fractions collected from ambient air (IARC, 2016; U.S. EPA, 2009). New studies involving $PM_{2.5}$ exposure published since the 2009 PM ISA also provide evidence of the presence of mutagenic agents (Lemos et al., 2016; Traversi et al., 2014; de Rainho et al., 2013; Rainho et al., 2013; Lemos et al., 2012; Singla et al., 2012; Traversi et al., 2011; Kawanaka et al., 2008; Traversi et al., 2008).

10.2.2.2 DNA Damage

10.2.2.2.1 Toxicological Evidence

In addition to *Salmonella* studies that evaluated mutagenicity, new reports that measured other effects relevant to genotoxicity and carcinogenicity as a result of $PM_{2.5}$ exposures have been published. Many of these studies used a variety of in vitro assays including cell-free and cell culture systems that are designed to identify specific cellular endpoints. For example, the comet assay measures DNA single- and double-strand breaks and can be adapted to identify the presence of apurinic and apyrimidinic (together noted as AP) sites by the introduction of alkaline conditions, and certain types of damaged bases, including oxidized bases, through the additional use of lesion-specific endonucleases (Collins et al., 2008). Several other assays to identify the presence of oxidative stress after $PM_{2.5}$ exposures have also been used in new studies. The relevance of data generated in the comet assay is supported by the fact that oxidative stress is one of the key characteristics of carcinogens (Smith et al., 2016).

The presence of reactive oxygen species (ROS) in a cell is a consequence of normal physiological processes, however oxidative stress, which is the imbalance between the generation of ROS and the protective mechanisms by which ROS are detoxified or ROS-induced damage is repaired, has been associated with the development of several health effects including cancer. ROS and ROS-induced lipid peroxidation products interact with DNA to form DNA lesions such as 7,8-dihydro-8-oxoguanine (8-oxoG), thymine glycol, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPy), etheno-DNA adducts, and malondialdehyde DNA adducts (Smart et al., 2008). The presence of these lesions can lead to the introduction of fixed mutations into the genome after incorrect repair of the damaged base or replication past the base by low fidelity DNA polymerases. The potential for oxidative stress to result in mutagenesis is underscored by the DNA repair mechanisms that have evolved to protect the genome from mutagenesis caused by these lesions. Increased 8-oxoG levels, one of the most widely studied lesions, has been demonstrated to result in spontaneous tumorigenesis in MTH1-deficient mice (Tsuzuki et al., 2001).

Since the 2009 PM ISA, several new studies have been published to identify the potential for oxidative stress resulting from exposure to $PM_{2.5}$. They have focused primarily on evaluating the oxidative potential of PM in acellular in vitro assays, as well as the capability of PM to induce oxidative stress in cultured cells. Because an important source of oxidative stress is inflammation, one new study measured in vitro inflammatory responses to $PM_{2.5}$ exposure. Evidence for inflammation at the organ and system level resulting from $PM_{2.5}$ exposure is described in Chapters 4 and 5.

Collectively, results from the in vitro studies demonstrate that damage to DNA bases and DNA strands can occur due to exposure to $PM_{2.5}$ in these systems and that production of ROS may contribute to that damage. As with *Salmonella* assay results, the findings are limited by the well understood caveats that apply to many in vitro model systems, including uncertainty regarding the relationship between measures of molecular markers and in vivo outcomes. As with the *Salmonella* assay, PM processing after

collection and use of extracted material in many studies may result in PM that is not representative of that in ambient air and/or alter its toxicity. For example, Turner et al. (2015) investigated how the use of EOM from collected particles may affect results for a suite of in vitro toxicity tests. The authors reported that the use of EOM from diesel exhaust particles (DEP) induced greater biological responses than intact DEP in suspension. They also evaluated the effect of cell type and observed that, in general, human premacrophage monocyte (GDM-1) cells were more sensitive than A549 cells.

Several studies measured DNA damage after exposure to ambient $PM_{2.5}$ (Lemos et al., 2016; Danielsen et al., 2011; Oh et al., 2011; Bonetta et al., 2009) and DEP (Dumax-Vorzet et al., 2015; Jalava et al., 2015; Gualtieri et al., 2011) using the comet assay. Although the variety of PM preparation and comet assay methods make direct comparisons difficult, the results suggest that exposure of cultured cells in vitro to $PM_{2.5}$ extracted material and/or suspensions can result in DNA damage. Oh et al. (2011) collected $PM_{2.5}$ from a traffic area in Suwon City, South Korea that was located approximately 20 miles south of Seoul and exposed human bronchial epithelial (BEAS-2B) cells to the organic crude extracted (CE) fraction as well as fractions of the CE that were separated by acid-base partitioning. Using the alkaline comet assay, the authors identified increased damage compared to control in the CE as well as the aliphatic, aromatic, and slightly polar fractions ($p < 0.01$). Repetition of the same assay with the addition of several different oxidant modulators rescued the damage to some extent in all cases, suggesting that the observed damage was, in part, the result of oxidative stress. Further, the authors also assessed the presence of specific lesions through the addition of formamidopyrimidine DNA glycosylase (FPG) and endonuclease III which can detect the presence of oxidized bases and some alkylation damage. For these experiments, increased damage was observed compared with controls in the CE as well as the fractions noted above ($p < 0.01$), providing support for the hypothesis that $PM_{2.5}$-induced oxidative stress can result in DNA damage.

These findings are supported by others (Danielsen et al., 2011; Gualtieri et al., 2011). Danielsen et al. (2011) detected DNA damage in A549 and human monocyte (THP-1) cells after exposure to PM (collection efficiency of 60–80% between 0.2 and 0.8 μm; upper cut point of 2.3 μm) suspension collected from two sites near Slagslunde, North Zealand, Denmark and confirmed the capability of the collected PM to generate ROS in other acellular and cell culture-based assays. Throughout their results, however, statistically significant increases ($p < 0.05$) were frequently observed only after exposure to suspension concentrations of greatest magnitude. In the study by Gualtieri et al. (2011), exposure to $PM_{2.5}$ suspension from PM collected near Milan, Italy resulted in an increase in DNA damage ($p < 0.05$) in BEAS-2B cells compared to controls.

In contrast to the findings by Danielsen et al. (2011) and Gualtieri et al. (2011), Jalava et al. (2015) did not observe DNA damage after exposure to fine PM suspensions. In this study, the authors exposed mouse macrophages (RAW 264.7) to $PM_{10-2.5}$, $PM_{2.5-1}$, $PM_{1-0.2}$, and $PM_{0.2}$ suspensions collected at Nanjing University in China and measured DNA damage using the alkaline comet assay. Although the

authors noted an increase in damage following some exposures to PM of other size fractions, there was no change in the damage measured in suspensions of $PM_{2.5-1}$ or $PM_{1-0.2}$ compared to controls.

Bonetta et al. (2009) also demonstrated the capability of PM extract exposure to result in DNA damage in the comet assay and observed that the amount of damage measured can vary with sampling location, which is consistent with similar findings in *Salmonella* assay studies. Using aqueous and organic extracts from $PM_{2.5}$ collected at urban, highway, and industrial sites near Alessandria, Italy, DNA damage was measured in human lung epithelial (A549) cells with the comet assay. The authors reported that exposure to organic extracts from $PM_{2.5}$ collected from all three sites resulted in an increase in damage using the alkaline comet assay compared to controls ($p < 0.001$). The increase was greatest for exposure to the highway site $PM_{2.5}$ organic extracts (p-value not provided). Exposure to aqueous extracts resulted in an increase in damage compared to control ($p < 0.001$) using the FPG-modified alkaline comet assay for $PM_{2.5}$ collected from the industrial site only.

Wessels et al. (2010) demonstrated that DNA damage after exposure to subfractions of $PM_{2.5}$ can vary with sampling location. To represent and compare diverse PM mixture profiles, the authors collected PM from four locations including a rural location at a beach on the west coast of Ireland and three urban locations in Birmingham, U.K. that varied in the extent to which vehicle traffic would contribute to the PM mixture sampled. Five size fractions were collected. $PM_{2.5}$ was collected in four fractions of particles with aerodynamic diameters of <0.5, 0.5−0.95, 0.95−1.5, and 1.5−3 μm. The fifth fraction comprised particles with diameters in the range of 3−7 μm. To evaluate the genotoxicity of aqueous PM suspensions, cultured A549 cells were used in the FPG-modified comet assay. The authors generally observed greater amounts of DNA damage after exposure to urban roadside PM suspensions compared to exposure to PM of equal mass collected from the rural site ($p < 0.1$) for size fractions with aerodynamic diameters of <0.5, 0.95−1.5, and 1.5−3 μm. This contrasts with the 3−7 μm fraction for which there was not a significant difference in the amount of damage induced after exposure to PM collected from any of the urban locations compared to that collected from the rural location. The variation in damage between size fractions was also examined. After adjusting for sampling site, the amount of DNA damage induced by extracts of PM from different particle size fractions was similar.

Borgie et al. (2015b) compared the effects of exposure to intact ambient $PM_{2.5}$ with aerodynamic diameters between 0.3 and 2.5 μm (described as $PM_{2.5-0.3}$) collected from an urban site in Beirut, Lebanon to that collected from a rural site in Byblos, Lebanon which is located 35 km from Beirut. The authors measured phosphorylated H2AX (γ-H2AX), a marker of DNA double strand breaks (DSB), in cultured BEAS-2B cells. Exposure to$PM_{2.5}$ collected from the urban location increased double-strand breaks at both low and high concentrations (3 and 12 $\mu g/cm^2$). In contrast, exposure to $PM_{2.5}$ collected from the rural location induced breaks only at the high concentration (12 $\mu g/cm^2$) only ($p < 0.05$), indicating that the $PM_{2.5}$ collected from the urban location had greater DNA damaging potency than that collected from the rural location.

The induction of oxidative stress after exposure to ambient $PM_{2.5}$ and DEP extracts and suspensions in cell culture demonstrated by comet assay results has been supported by studies that have used other in vitro methods to measure oxidative stress (Dumax-Vorzet et al., 2015; Miousse et al., 2015; Mirowsky et al., 2015; Gordon et al., 2013). Mirowsky et al. (2015) collected $PM_{2.5}$ as well as $PM_{10-2.5}$ from two rural and three urban sites in California and generated aqueous suspensions of both soluble and insoluble material. Using cultured human pulmonary microvasculature endothelial (HPMEC-ST11.6R) cells, they measured ROS with 2′,7′-dichlorofluorescein diacetate (DCFH-DA). DCFH-DA, after removal of the acetate groups by cellular esterases, can be oxidized to highly fluorescent DCF that can then be used to quantify the amount of intracellular ROS. The results identified two variables. That is, both the size fraction and location at which the PM was collected can affect the amount of intracellular ROS generated after exposure to aqueous PM suspension. Suspensions of $PM_{2.5}$ collected at urban sites were characterized by less ROS activity than those of $PM_{10-2.5}$ ($p < 0.001$). The same outcome was not observed, however, after exposure to $PM_{2.5}$ and $PM_{10-2.5}$ suspensions from the rural sites because the ROS activity generated by both was similar. When comparing the same size fractions between urban and rural sites, no differences were reported between sites for the $PM_{2.5}$ suspensions, whereas greater ROS activity was observed in experiments with $PM_{10-2.5}$ from the urban sites than $PM_{10-2.5}$ collected at the rural sites (p-value not provided).

Additional studies were identified that also used the DCFA-FA assay to assess intracellular ROS after exposure to PM (Dumax-Vorzet et al., 2015; Gordon et al., 2013). Gordon et al. (2013) exposed BEAS-2B and HBEpC cells to suspensions of size-fractionated PM from ambient air collected from five diverse sampling locations across the U.S. The PM size fractions collected were described as $PM_{2.5-0.2}$, $PM_{10-2.5}$, and $PM_{0.2}$. Like several other findings already highlighted, the authors reported variation in ROS production because of sampling site, season, and particle size. The report also noted that exposure to the $PM_{2.5}$ resulted in ROS production that was less than that of either $PM_{10-2.5}$ or UFP on an equal mass exposure when sampling locations were combined. Dumax-Vorzet et al. (2015) used cultured mouse embryonic fibroblasts (MEFs) in the DCFA-DA assay in addition to an acellular plasmid scission assay to estimate ROS after exposure to DEP suspension. The authors noted a dose-dependent increase in ROS (p-value not provided) using both methods.

Studies that used in vitro methods other than DCFA-FA to evaluate ROS or measured other endpoints that are relevant to oxidative stress have also been published. A change in superoxide was not detected in a study by Miousse et al. (2015) using dihydroethidium oxidation after exposure to aqueous extracts from $PM_{2.5}$ collected at an underground parking deck, but an increase in catalase expression ($p < 0.01$) was noted by the authors. Mirowsky et al. (2015) evaluated infiltrating polymorphonuclear cells (PMNs) as inflammation and ROS generated by PMNs in response to PM exposure has also been proposed as a pathway that may result in genotoxicity. The authors compared the effect of exposure on the percent of PMNs in lavage fluid for the various sampling locations and PM size fractions using oropharyngeal aspiration of aqueous PM suspension exposure in mice (FVB/N). Except for one rural location, the increase in percentage of PMNs after exposure to $PM_{2.5}$ suspensions were less than that after

exposure to $PM_{10-2.5}$ ($p < 0.001$). Upregulation of genes involved in antioxidant defense, i.e., the Phase 2 enzymes, were also observed in different in in vitro systems after $PM_{2.5}$ exposure. Borgie et al. (2015b), as described above, found increased gene expression of NQO1 in BEAS-2B cells.

In addition to in vitro studies, one in vivo study examined DNA damage. Exposure of male C57BL/6 mice to concentrated ambient $PM_{2.5}$ ($PM_{2.5}$ CAPs) in Chicago, IL resulted in an increase in 8-oxoG positive nuclei in lung tissue ($p < 0.01$) (Soberanes et al., 2012). This finding provides evidence of oxidative DNA damage in lungs following $PM_{2.5}$ exposure.

In summary, numerous in vitro studies conducted in cultured cells provide evidence of DNA damage, measured as single- and double-strand breaks, following exposure to suspended $PM_{2.5}$ or $PM_{2.5}$ extracts. Increased ROS production was also found in cellular assays. These results indicate that exposure to $PM_{2.5}$ induces oxidative stress, one of the identified characteristics of a carcinogen (Smith et al., 2016). Additionally, there is evidence of a direct relationship between oxidative stress and DNA damage. In an in vivo study, $PM_{2.5}$ CAPs inhalation resulted in oxidative DNA damage in lungs.

10.2.2.2.2 Evidence from Controlled Human Exposure Studies

Controlled human exposure studies have evaluated various markers relevant to DNA damage. Hemmingsen et al. (2015) reported mostly negative findings for DNA damage and oxidative stress from a controlled, cross-over, repeated measures human exposure study carried out in central Copenhagen, Denmark. In this study, overweight, older adults were exposed for 5 hours in chambers with and without high efficiency particulate adsorption filters. Peripheral blood mononuclear cells collected immediately before and after the exposure were negative for change from controls for several endpoints evaluated. These include ROS production, DNA strand breaks, oxidized DNA bases, and mRNA expression of CCL2, IL8, TNF, HMOX1, and OGG1. The only positive association identified was between FPG sensitive sites and exposure to urban air although it failed to reach statistical significance.

Another controlled human exposure study by Liu et al. (2015) measured malondialdehyde (MDA) in blood and urine and 8-oxo-dG in urine. The former is a lipid peroxidation product capable of reacting with DNA bases, whereas the latter is excreted after oxidized dGTP molecules in cellular dNTP pools used for nuclear and mitochondrial DNA replication throughout the cell are acted upon by MTH1 followed by 8-oxo-dGMPase in the process of dNTP pool sanitization. In this single-blind randomized crossover study, nonsmoking adults were exposed for 130 minutes to $PM_{10-2.5}$, $PM_{2.5}$, and UFP CAPs drawn from a downtown street in Toronto, Canada. Participant blood and urine were collected before exposure and after exposure at two-time points (1-hour, 21 hours). Positive associations between urinary MDA concentrations and $PM_{2.5}$ CAPs were reported for both time points (1-hour post-exposure: $p < 0.05$; 21 hours post-exposure $p < 0.1$). Urinary creatinine was used to normalize biomarker concentrations. No association was observed between blood MDA concentration and concentration of $PM_{2.5}$.

10.2.2.2.3 Epidemiologic Evidence

Several recent studies have examined a variety of molecular and cellular markers often associated with DNA damage. Study characteristics including $PM_{2.5}$ concentrations, study population, and exposure assignment approach for the studies that examined long-term $PM_{2.5}$ exposure and DNA damage are detailed in Table 10-1.

Table 10-1 Study specific details and $PM_{2.5}$ concentrations from recent studies that examined DNA damage.

Study Years	Location Population	Endpoints	Mean Concentration µg/m^3	Exposure Assessment
†Rossner et al. (2013b) (Winter and Summer 2009; Winter 2010)	Prague and Ostrava, Czech Republic (Prague: 61–65, nonsmoking policemen; Ostrava: 98–149; policemen, office workers, and volunteers)	B[a]P-like DNA adducts	Winter 2009: Prague: 13.8 Ostrava: 40.0 Summer 2009: Prague: 13.3 Ostrava: 12.0 Winter 2010: Prague: 42.6 Ostrava: 78.9	Personal monitoring for 48 h in each month, ambient concentrations measured up to 90 days before personal sampling
†Li et al. (2014) (2009–2010)	Shanghai, China (107 traffic policemen, 101 office workers)	BPDE-DNA adducts	Traffic policemen: 115.4 Office workers: 74.9	Personal 24-h concentrations
†Chu et al. (2015) (Not reported)	Zhuhai, Wuhan, and Tianjin China (307 subjects)	% tail DNA (comet assay)	Zhuhai: 68.4[a] Wuhan: 115.0[a] Tianjin: 146.6[a]	Personal 24-h concentrations
†Ma et al. (2015) (2013)	Shenyang, China (16 traffic policemen, 16 nonfield traffic policemen)	% tail DNA (comet assay)	Traffic policemen: 162.7 Nonfield traffic policemen: 51.5	2-week monitoring (April 8–19, 2013) campaign at traffic sites and indoor offices

B[a]P = benzo[a]pyrene; BPDE = (+) -enantiomer of antibenzo[a]pyrene 7,8-diol-9,10-epoxide; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

[a]Median concentration.

†Studies published since the 2009 PM ISA.

Rossner et al. (2013b) examined bulky B[a]P-like DNA adducts in study populations in two Czech Republic cities, Prague and a more polluted city (i.e., higher concentrations of not only PM but other pollutants as well), Ostrava. Whereas the study population in Prague consisted of only nonsmoking policeman, the study population in Ostrava was comprised of policeman, office workers, and volunteers.

This resulted in two different types of study populations where one consisted of individuals that may have smoked. Smoking status was not specifically adjusted for in the statistical models, but measures of cotinine in the blood, a proxy for tobacco smoke exposure was included as a covariate. This study found a higher number of B[a]P-like adducts in people that resided in Ostrava in association with $PM_{2.5}$ concentrations ($\beta = 0.002$ [95% CI: 0.002, 0.003]). These results are consistent with Li et al. (2014) in a study conducted in Shanghai, China that examined B[a]P-like adducts in a population of nonsmoking men that were traffic policemen or office workers. Using $PM_{2.5}$ concentrations collected through personal monitoring the 24-hours preceding biological sample collection, the authors observed an overall increase in BPDE-DNA adducts (0.8% [95% CI: 0.4, 1.2]), which was driven by the exposure group (1.2% [95% CI: 0.6, 1.5]) consisting of traffic policeman with limited evidence of an increase (0.1% [95% CI: 0.02, 0.23]) in the control group (i.e., office workers).

A study conducted in a cohort from three Chinese cities (Zhuhai, Wuhan, and Tianjin) broadly examined $PM_{2.5}$-modulated DNA damage by focusing on tail DNA and whether specific genetic polymorphisms modify the effect (Chu et al., 2015). Using $PM_{2.5}$ data from a personal monitoring campaign, Chu et al. (2015) reported evidence of a weak positive association between $PM_{2.5}$ concentrations and percentage of tail DNA from peripheral blood samples ($\beta = 0.001$ [95% CI: 0.000, 0.002]). These results are consistent with some of the results from Ma et al. (2015) in a study of DNA damage conducted in Shenyang consisting of traffic and nonfield traffic policemen. The authors graded the extent of DNA damage on a scale of 1 to 3, where 1 and 2 represented DNA damage <40% and 3 >40% damage. For DNA damage graded 1 and 2, Ma et al. (2015) did not observe a difference in the level of DNA damage between policemen exposed to high and low $PM_{2.5}$ concentrations. However, when examining Grade 3, there was a much larger percent of DNA damage in the traffic policemen compared to the nonfield policemen.

10.2.2.2.4 Summary

In summary, several lines of evidence provide support for a relationship between exposure to $PM_{2.5}$ and DNA damage. in vitro toxicological studies demonstrate that damage to DNA bases and DNA strands can occur after exposure to $PM_{2.5}$ in these systems and that production of ROS may contribute to that damage. An animal inhalation study (Soberanes et al., 2012) and a controlled human exposure study (Liu et al., 2015) also provide evidence of oxidative DNA damage. These findings are supported by epidemiologic studies that demonstrate DNA damage in association with $PM_{2.5}$ concentrations (Chu et al., 2015; Ma et al., 2015). In addition, epidemiologic studies indicated a larger percentage of B[a]P-like DNA adducts in people exposed to higher $PM_{2.5}$ concentrations (Li et al., 2014; Rossner et al., 2013b).

10.2.2.3 Cytogenetic Endpoints

10.2.2.3.1 Toxicological Evidence

New in vitro studies also demonstrated the presence of chromosomal abnormalities using the cytokinesis block micronucleus assay (CBMN) after exposure to $PM_{2.5}$ (Lemos et al., 2016; Oh et al., 2011). The CBMN assay detects acentric chromosome fragment loss and whole chromosome loss resulting from clastogenic and aneugenic agents, respectively (Kirsch-Volders et al., 2003). Lemos et al. (2016) exposed Chinese hamster lung fibroblasts (V79) to EOM material from $PM_{2.5}$ collected near a petrochemical complex in Triunfo, Brazil. In total, 23 results were reported comprising exposures to two concentrations of samples collected in two locations over several different seasons. Of those 23 results, increases over controls were noted for only three ($p < 0.05$). The remaining 20 results were negative for increases. Oh et al. (2011) also measured micronuclei and reported results consistent with comet assay results reported in the same study (see Section 10.2.2.2). That is, increases in micronuclei in the aliphatic, aromatic, and slightly polar fractions as well as the highest doses of CE compared to controls ($p < 0.01$) were observed in organic extracts from $PM_{2.5}$ collected near Seoul, Korea and this damage was prevented by the addition of ROS scavengers, as was the case for the comet assay results.

10.2.2.3.2 Epidemiologic Evidence

Recent studies have examined cytogenetic endpoints such as chromosomal aberrations and micronuclei. Study characteristics including $PM_{2.5}$ concentrations, study population, and approaches to exposure assignment are detailed in Table 10-2.

Table 10-2 Study specific details and $PM_{2.5}$ concentrations from recent studies that examined cytogenetic endpoints.

Study Years	Location Population	Endpoints	Mean Concentration µg/m^3	Exposure Assessment
†Rossner et al. (2011) (Feb–May 2007)	Prague, Czech Republic (59 city policemen)	FG/100; %AB.C; ace	Feb: 26.1 May: 28.4	Personal monitoring for 48 h in each month, ambient concentrations measured up to 90 days before personal sampling
†Rossner et al. (2013a) (Winter and Summer 2009; Winter 2010)	Prague and Ostrava, Czech Republic (Prague: 61–65, nonsmoking policemen; Ostrava: 98–149; policemen, office workers, and volunteers)	FG/100; %AB.C; ace; MN/1,000 BC	Winter 2009: Prague: 13.8 Ostrava: 40.0 Summer 2009: Prague: 13.3 Ostrava: 12.0 Winter 2010: Prague: 42.6 Ostrava: 78.9	Personal monitoring for 48 h in each month, ambient concentrations measured up to 90 days before personal sampling
†Ceretti et al. (2014) (Winter 2012 and 2013)	Brescia, Italy (RESPIRA, 181 children, 3–6 yr old)	% MN; % nuclear buds; % binucleated cells; % basal cells; % condensed chromatic cells; % karyorrhectic cells; % pyknotic cells; % karyolitic cells; % without nucleus cells	Same day[a]: 24–96 1 week: 32.8–93.1 2 weeks: 40.1–82.6 3 weeks: 41.7–70.1	Ambient concentrations obtained from Regional Agency for Environmental Protection database
†O'Callaghan-Gordo et al. (2015) (Feb 2009–2010)	Crete, Greece (136 mother-child pairs)	MN/1,000 BC	14.4[b]	2 week monitoring at 40 sites used as input to LUR model based on ESCAPE protocol as detailed in (Beelen et al., 2013); (Eeftens et al., 2012b) to maternal home address

FG/100 = genomic frequency of translocations; %AB.C = percentage of aberrant cells; ace = number of acentric fragments; MN/1,000 BC = frequency of micronuclei per 1,000 binucleated cells; % MN = percent of micronuclei; RESPIRA = Italian acronym for Rischio ESPosizione Inquinamento aRia Atmosferica study.

[a]Range of mean concentrations across days of biological sampling, same day and 1–3 weeks prior to biological sampling.

[b]Median concentration.

†Studies published since the 2009 PM ISA.

Recent studies conducted in the Czech Republic that examined the relationship between $PM_{2.5}$ exposure and cytogenetic effects did not report clear evidence of associations. Rossner et al. (2011), in a study of nonsmoking policemen working more than 8 hours outdoors per day in Prague, reported no association between $PM_{2.5}$ concentrations measured by ambient monitors in the 2-days prior to personal sampling and the genomic frequency of translocations, percentage of aberrant cells, or the number of acentric fragments. However, when examining different time windows by extending out to longer lags, there was evidence of a positive association between $PM_{2.5}$ concentrations in the 15−28 days prior to personal sampling and the number of acentric fragments ($\beta = 0.64$ [95% CI: 0.05, 1.24]). This initial study by Rossner et al. (2011) that focused on Prague was expanded upon to include participants that were defined as living in a more polluted city, Ostrava (Rossner et al., 2013a). As detailed in Section 10.2.2.2, the study populations between Prague and Ostrava differed in that individuals in Ostrava may have smoked. Similar to Rossner et al. (2013b), smoking status was not specifically adjusted for in Rossner et al. (2013a), but measures of cotinine in the blood, a proxy for tobacco smoke exposure was included as a covariate. Rossner et al. (2013a) examined the same markers of chromosomal aberration as Rossner et al. (2011), but also examined the number of micronuclei. When comparing the stable chromosomal aberrations (i.e., genomic frequency of translocations, percentage of aberrant cells, or the number of acentric fragments), the authors observed relatively similar results in both study locations in the 2-days prior to personal sampling even though the $PM_{2.5}$ concentrations were much higher in Ostrava. However, when examining longer lags of exposure (i.e., 1−14 days prior to sampling) there was evidence of a positive association between $PM_{2.5}$ concentrations and the percentage of aberrant cells in Prague (OR = 2.43 [95% CI: 1.26, 4.68], increment not specific). An examination of the frequency of micronuclei found a lower percentage in Ostrava ($\beta = -0.032$ [95% CI: −0.042, −0.022]) than Prague ($\beta = -0.074$ [95% CI: −0.114, −0.034]).

Additional studies conducted in Italy and Greece examined associations between $PM_{2.5}$ and cytogenetic endpoints with a focus on micronuclei frequency. Ceretti et al. (2014) as part of the RESPIRA study, examined cytogenetic endpoints in exfoliated buccal cells of children residing in Brescia, Italy. The study focused on air pollution concentrations during the winter months because that period of the year has higher concentrations of pollutants, including $PM_{2.5}$. The authors reported no evidence of a positive association between $PM_{2.5}$ concentrations assessed on the same day or during the 1, 2, or 3 weeks prior to biological sample collection and micronuclei frequency. However, there was some evidence of increases in the frequency of nuclear buds, binucleated cells, basal cells, and condensed chromatin cells with $PM_{2.5}$ concentrations in the 1 week prior to biological sample collection. O'Callaghan-Gordo et al. (2015) took a different approach to examining micronuclei frequency in children by focusing on whether a higher micronuclei frequency in pregnant women attributed to air pollution exposure led to higher micronuclei frequencies in children at the time of birth. As part of the Rhea mother-child cohort, O'Callaghan-Gordo et al. (2015) reported positive associations between $PM_{2.5}$ concentrations over the entire pregnancy and micronuclei frequency in maternal (RR = 1.5 [95% CI: 1.0, 2.3]), but not cord, blood (RR = 0.97 [95% CI: 0.63, 1.50]). However, when stratifying by smoking status, an association larger in magnitude was observed in smoking mothers (RR = 1.7 [95% CI: 0.95, 3.1]) compared to nonsmokers (RR = 1.4 [95%

CI: 0.80, 2.5]), but 95% confidence intervals crossed the null for both. Additionally, the association between $PM_{2.5}$ and micronuclei frequency was found to be increased among women with a lower intake of vitamin C during pregnancy (i.e., <85 ng/day).

10.2.2.3.3 Summary

In summary, there is some support for a relationship between exposure to $PM_{2.5}$ and cytogenetic effects. Toxicological studies demonstrate chromosomal abnormalities and micronuclei formation after exposure to $PM_{2.5}$ in in vitro systems and suggest that production of ROS may contribute to the damage (Oh et al., 2011). Epidemiologic studies provide weaker evidence of cytogenetic effects in association with exposure to $PM_{2.5}$; however, there is initial evidence that micronuclei frequency may be correlated with the intake of an antioxidant nutrient (O'Callaghan-Gordo et al., 2015).

10.2.2.4 Other Markers

10.2.2.4.1 Toxicological Evidence

Studies have also evaluated several other molecular and cellular endpoints that are relevant to carcinogenesis. Many of these studies describe events important to the DNA damage response and gene expression that may be relevant to cancer initiation and progression. Expression of genes that participate in PAH biotransformation have been commonly measured in new studies and include AhR, AhRR, ARNT, Cyp1A1 and Cyp1B1 (Yoshizaki et al., 2016; Borgie et al., 2015b; Gualtieri et al., 2011; Oh et al., 2011). Biotransformation may result in the production of PAH metabolites capable of reacting with DNA to form DNA adducts. When DNA repair is absent or ineffective, the formation of DNA adducts may be processed by the cell to mutations.

Borgie et al. (2015b) compared the effects of exposure to intact ambient $PM_{2.5}$ with aerodynamic diameters between 0.3 and 2.5 μm (described as $PM_{2.5-0.3}$) collected from an urban site in Beirut, Lebanon to that collected from a rural site in Byblos, Lebanon which is located 35 km from Beirut. The authors measured AhR, ARNT, AhRR, CYP1A1, and CYP1B1 gene expression in cultured BEAS-2B cells. A general pattern was observed for measurements of CYP1A1 and AhRR expression. That is, after exposure to $PM_{2.5}$ collected from the urban location, increases in expression were observed compared to controls after exposure to both low and high concentrations (3 and 12 $\mu g/cm^2$). In contrast, $PM_{2.5}$ collected from the rural location resulted in an increase compared to control for the high concentration exposure (12 $\mu g/cm^2$) only ($p < 0.05$), indicating that the $PM_{2.5}$ collected from the urban location may possess greater potency than that collected from the rural location. Some increases were also observed for CYP1B1 expression, whereas results were generally negative for AhR and ARNT expression. The finding of increased CYP1A1 expression was confirmed by Oh et al. (2011), discussed above. They estimated

CYP1A1 activity using the ethoxyresorufin-O-deethylase (EROD) assay and reported an increase compared with controls in the total extract as well as the aromatic fraction ($p < 0.01$). Gualtieri et al. (2011) also measured gene expression. They too noted an increase in Cyp1A1 ($p < 0.0001$), Cyp1B1 (p-value not provided) and AhRR (p-value not provided) expression, similar to both Borgie et al. (2015b) and Oh et al. (2011). AhR (p-value not provided) and ARNT (p-value not provided) expression decreased after exposure of BEAS-2B cells to $PM_{2.5}$. Dumax-Vorzet et al. (2015) also measured Cyp1A1 expression; however, the authors did not observe evidence of an increase in Cyp1A1 mRNA after exposure to DEP particle suspension. Because the authors did observe an increase in ROS in the same study, they concluded that Cyp1A1 activity was not the source of the increased ROS.

mRNA expression of some of the same genes detailed in the previous paragraph were measured in an animal study by Yoshizaki et al. (2016). In this study, mRNA from nasal epithelium was quantified for AhR, Cyp1A1, Cyp1A2, Cyp1B1, Erβ-1, and Erβ-2 in male and female BALB/c mice exposed to $PM_{2.5}$ CAPs in São Paulo, Brazil. After exposure, only two changes were reported. Cyp1B1 mRNA expression was increased in exposed female ($p = 0.01$), but not male mice compared with animals exposed to ambient air, and Erβ-2 mRNA expression was decreased in exposed female ($p = 0.007$), but not male mice compared with animals exposed to ambient air. There was not an increase in mRNA for the other four genes evaluated in male or female mice. The authors also measured AhR- and Erβ-positive nuclei in nasal epithelium cells. They observed an increase in the percent of AhR-positive nuclei in $PM_{2.5}$-exposed female ($p = 0.044$) but not male mice compared with controls, and a decrease in the percent of Erβ-positive nuclei in female, but not male mice compared with mice exposed to ambient air.

In addition, one study evaluated the effect of $PM_{2.5}$ exposure on telomerase. Telomerase is a protein that adds telomere repeat sequences to the ends of chromosomes. This is one way in which cells avoid senescence and arrested cell division. Telomerase can play a role in cancer development by conferring cellular immortality. Borgie et al. (2015b) reported increased telomerase activity in cultured BEAS-2B cells exposed to $PM_{2.5}$.

10.2.2.4.2 Evidence from Controlled Human Exposure Studies

Hemmingsen et al. (2015) reported negative findings for mRNA expression of CCL2, IL8, TNF, HMOX1, and OGG1 in a controlled, cross-over, repeated measures human exposure study carried out in central Copenhagen, Denmark. In this study, overweight, older adults were exposed in chambers with and without high efficiency particulate adsorption filters. Peripheral blood mononuclear cells collected immediately before and after the exposure were negative for change from controls for several endpoints evaluated.

10.2.2.4.3 Epidemiologic Evidence

In addition to examining specific changes in the genome that could lead to cancer, an additional study focused on whether $PM_{2.5}$ exposure resulted in differential expression of genes related to a specific health outcome, such as cancer (Chu et al., 2016). Within the TrIPS study, a panel of 63 nonsmoking white men were selected to examine the relationship between gene expression and long-term traffic-related pollution exposure (i.e., $PM_{2.5}$, EC, and OC). Long-term $PM_{2.5}$ exposure was defined as the exposure during the first and last work shift within a week. To focus the analysis on a collection of genes that may influence a health outcome, the authors applied Gene Set Enrichment Analysis (GSEA) to examine gene specific networks. The GSEA analysis identified 44 genes that were previously related to various cellular and biological processes. Chu et al. (2016) then used GeneMANIA network analysis to examine the inter-relationship among this core set of 44 genes. The authors found evidence that long-term exposure to traffic-related pollutants, including $PM_{2.5}$, increased the expression of five genes (ACP1, HSP90AA1, LEF1, MLH1, and RBM5) that are common in cancer pathogenesis.

10.2.2.4.4 Summary

Studies in cultured cells in vitro and in an animal model have demonstrated the upregulation of genes involved in PAH biotransformation following exposure to suspended $PM_{2.5}$ or $PM_{2.5}$ extracts. These results indicate that $PM_{2.5}$ contains electrophilic species, one of the identified characteristics of a carcinogen (Smith et al., 2016). $PM_{2.5}$ exposure also increased telomerase activity in vitro. This result indicates that $PM_{2.5}$ may promote cellular immortalization, another of the characteristics of a carcinogen. Epidemiologic studies link exposure to $PM_{2.5}$ with the upregulation of several genes that may be involved in cancer pathogenesis.

10.2.2.5 Summary of Genotoxicity

Studies published since the completion of the 2009 PM ISA (U.S. EPA, 2009) provide a broader evaluation of the relationship between $PM_{2.5}$ exposure and mutagenicity, DNA damage, cytogenetic effects, and other markers of genotoxicity. The importance of *Salmonella* assay results is that positive results demonstrate the presence of species capable of inducing mutations. It can identify the presence of species that can result in mutations as the result of direct interactions with DNA as well as those that require metabolic activation. Because the most widely accepted theory of cancer etiology is the accumulation of mutations in critical genes, the presence of mutagens within $PM_{2.5}$ and the mutagenicity of organic extracts of $PM_{2.5}$ provide biological plausibility for observations made in epidemiologic studies. Further, results can suggest the presence of certain species such as nitro-polycyclic aromatic compounds (nitro-PAHs). The *Salmonella* assay, however, does not capture the complex biological in vivo activity of human cells, tissues, and other processes or systems of increasing biological

organization. Therefore, although exposure to mutagens present in $PM_{2.5}$ clearly could result in the introduction of mutations that could lead to initiated cells in vivo, strictly interpreted, the results from *Salmonella* only provide evidence for the presence of species capable of inducing mutagenesis. Thus, it is also necessary to consider results from in vitro assays that use mammalian cell lines and in vivo animal studies to completely characterize the effects of PM exposure in humans.

Toxicological studies conducted in mammalian cell lines demonstrated damage to DNA bases, DNA strand breaks, oxidative stress, micronuclei formation, and chromosomal aberrations in response to $PM_{2.5}$ exposure. Upregulation of enzymes involved in antioxidant defense or biotransformation was also found. Dampening oxidative stress using inhibitors decreased DNA damage and micronuclei formation, supporting a role for oxidative stress in mediating genotoxicity (Oh et al., 2011). Although limited in number, some in vivo studies also examined DNA damage following $PM_{2.5}$ exposure. One study, using $PM_{2.5}$ CAPs collected in Chicago, found evidence of oxidative DNA damage in lung tissue (Soberanes et al., 2012). Controlled human exposure studies, including a study using $PM_{2.5}$ CAPs, also demonstrated oxidative DNA damage. A limitation of the collective body of in vitro evidence is that $PM_{2.5}$ was mainly collected overseas in locations with high pollution levels. A limitation of the in vivo evidence is that there are only a few studies. However, one of these found both evidence of oxidative DNA damage and methylation of the promotor region of a tumor suppressor gene in the lung (see also Section 10.2.3.1).

Epidemiologic studies examined a variety of biomarkers and collectively did not provide clear evidence of a relationship between any specific marker and $PM_{2.5}$ exposure. Although there was some evidence indicating a larger percentage of B[a]P-like DNA adducts in people exposed to higher $PM_{2.5}$ concentrations (Li et al., 2014; Rossner et al., 2013b), clear associations between $PM_{2.5}$ and various cytogenetic parameters were not observed in recent studies in the Czech Republic (Rossner et al., 2013b; Rossner et al., 2011). Only one study examined the association between $PM_{2.5}$ and micronuclei frequency in maternal blood and reported evidence of increased micronuclei frequency, specifically in women with low intake of vitamin C during pregnancy (O'Callaghan-Gordo et al., 2015). Those studies that examined DNA damage, by focusing on tail DNA, reported weak positive associations between personal $PM_{2.5}$ concentrations and percentage of tail DNA (Chu et al., 2015; Ma et al., 2015). Additionally, there is preliminary evidence that long-term $PM_{2.5}$ exposure may result in the differential expression of genes linked with cancer pathogenesis.

10.2.3 Epigenetic Effects

Epigenetic mechanisms regulate the transcription of genes without altering the nucleotide sequence of DNA. These mechanisms generally involve DNA methylation, histone modifications, chromatin remodeling, and changes in noncoding mRNA and nuclear organization and lead to alterations that may have long-term consequences or are heritable (Keverne and Curley, 2008; Jones and Baylin, 2007). DNA methylation and histone modifications, which include methylation, acetylation,

phosphorylation, ubiquitylation, and sumoylation, are known to be linked (Hitchler and Domann, 2007; Jones and Baylin, 2007). Numerous studies have identified epigenetic processes in the control of cancer (Foley et al., 2009; Gopalakrishnan et al., 2008; Jones and Baylin, 2007; Valinluck et al., 2004), embryonic development (Foley et al., 2009; Gopalakrishnan et al., 2008; Keverne and Curley, 2008), and inflammation and other immune system functions (Adcock et al., 2007).

Epigenetic modifications resulting in decreased expression of tumor suppressor genes and increased expression of transforming genes have been observed in human tumors (Valinluck et al., 2004). In general, transcription repression is associated with DNA methylation in promoter regions of genes. Cytosine methylation in CpG dinucleotides has emerged as an important, heritable epigenetic modification that can result in chromatin remodeling and decreased gene expression. Global changes in DNA methylation are also seen in cancer and hypomethylation is associated with genomic instability (Gopalakrishnan et al., 2008).

Growing evidence demonstrates the epigenetic effects of PM exposure, which is associated primarily with alterations in DNA methylation. In the 2009 PM ISA, there were a small number of epidemiologic studies that examined epigenetic effects, specifically methylation. DNA methylation is an epigenetic mechanism that regulates the proper expression of genetic information in a tissue-, cell-, and sex-dependent manner and controls the expression of tumor promotor and suppressor genes and of repetitive elements. Repetitive elements comprise up to ⅔ of mammalian genomes and are heavily methylated to prevent their aberrant transcription. Thus, repetitive element methylation levels have been used as surrogate biomarkers of global DNA methylation, which is linked to genomic instability and thus may contribute to the accumulation of mutations. A large subset of studies has evaluated the effect of PM exposure on this marker. In particular, research has focused on retrotransposons LINE-1 and Alu (SINE in mouse) and satellite DNA. Studies evaluated in the 2009 PM ISA found inconsistent evidence of an association between PM exposure and methylation of Alu and long interspersed nuclear element-1 (LINE-1) sequences, two sequences linked previously with global genomic DNA methylation. Recent epidemiologic studies further evaluated DNA methylation, and provide evidence for both hyper- and hypomethylation in response to $PM_{2.5}$ exposure. Both DNA hyper- and hypomethylation have been observed in malignant cells. Recent animal toxicological studies investigated epigenetic effects resulting from $PM_{2.5}$ exposure and provide evidence for methylation of a tumor promotor gene and alteration in noncoding mRNA.

10.2.3.1 Methylation of Tumor Suppressor Genes

Evidence that exposure to $PM_{2.5}$ results in the methylation of tumor promoter genes is provided by animal toxicological studies. Soberanes et al. (2012) measured molecular markers that have been associated with an increased risk of cancer in a high-risk smoking cohort. Using male C57BL/6 mice, the authors reported increased promotor methylation of p16 (CDNK2A), a tumor suppressor, and of matrix

metalloproteinase-2 (MMP-2) compared to controls ($p < .001$) in whole lung genomic DNA following inhalational exposure to $PM_{2.5}$ CAPs in Chicago, IL. The authors also reported an increase in DNA methyltransferase 1 (DNMT1) mRNA and protein ($p < 0.01$), but not DNMT3a or DNMT3b expression. Finally, they also noted an increase in 8-oxoG positive nuclei in lung tissue ($p < 0.01$), supporting the presence of ROS following $PM_{2.5}$ exposure. Alveolar epithelial cells exposed to the same $PM_{2.5}$ CAPs exhibited increased DNMT1 transcription and methylation of the p16 promotor; these effects were inhibited by treatment with an antioxidant targeted to mitochondria and by an inhibitor of JNK.

Another study using Wistar rats measured changes in p16CDNK2A (CDNK2A) and APC promoter methylation following $PM_{2.5}$ exposures of 4 hours to 28 days (Ding et al., 2016). Animals were exposed to ambient air at three sites in Zhejiang, China. Exposed rats were housed in cages roadside of a traffic tunnel and busy intersection; control rats were housed in cages at a university greenspace 0.5 mile from the nearest road. Although the authors made separate measurements for spring and autumn seasons, the DNA methylation was not different between the seasons, so seasonal data were analyzed together. The authors reported β-values and 95% confidence intervals for methylation of p16CDNK2A and APC promoters in peripheral blood and lung tissue after exposures of 4 hours and 7 days. The authors did not observe an association between $PM_{2.5}$ mass over exposures of these durations and p16CDNK2A promoter methylation in blood or lung tissue. An association was calculated for APC promoter methylation for only the 7-day exposure in lung tissue (0.009 [0.001, 0.019], $p = 0.046$). The study also reported associations after 14−28 days of exposure and note a positive exposure between $PM_{2.5}$ mass and p16CDNK2A promoter methylation in blood (0.037 [0.017, 0.057], $p = 0.001$) and lung tissue (0.011 [0.003, 0.019], $p = 0.011$), as well as APC promoter methylation in lung tissue (0.008 [0.002, 0.015], $p = 0.046$). The authors noted that methylation changes generally returned to levels comparable to controls after the longer 28-day exposures. The appreciable difference in environment between the exposure sites and plausible introduction of other stressors into the environment of the experimental animals elevates the uncertainty in the reported results.

10.2.3.2 Methylation of Repetitive Line Elements

10.2.3.2.1 Toxicological Evidence

In the experimental animal study discussed above using Wistar rats, Ding et al. (2016) also characterized global epigenetic changes represented by LINE-1 and Alu methylation after exposure to $PM_{2.5}$. Animals were exposed to low, medium, and high levels of traffic-related air pollution in Zhejiang, China. The authors reported β-values and 95% confidence intervals for methylation of LINE-1 in peripheral blood and lung tissue. They observed associations with 4-hour $PM_{2.5}$ exposure and decreased LINE-1 methylation in blood (−0.027 [−0.041, −0.013], $p = 0.003$) and lung (−0.041 [−0.049, −0.032], $p < 0.001$) tissues as well as with exposure for 7 days (blood: −0.064 [−0.104, −0.023], $p = 0.003$; lung:

−0.033 [−0.058, −0.008], $p = 0.012$). After 14 and 28 days, decreased LINE−1 methylation was associated with $PM_{2.5}$ exposure in the lung (−0.015 [−0.028, −0.002], $p = 0.024$). No associations were observed with Alu methylation. The authors do note that methylation changes generally returned to levels comparable to controls after the longer 28-day exposures.

Montrose et al. (2015) also investigated global DNA methylation in peripheral blood in a study of sled dogs residing in kennels in and near Fairbanks, AK. During Alaskan winters, severe temperature inversions result in elevated $PM_{2.5}$ concentrations in Fairbanks. Sled dogs housed at three kennels were recruited to participate. Average $PM_{2.5}$ mass was 90 μg/m^3 at Kennel A, 48 μg/m^3 at Kennel B, and 16 μg/m^3 at Kennel C. The authors did not identify any differences in the levels of global DNA methylation or percentage of methylated cytosine bases between the dogs from three kennels, and thus did not find an association between $PM_{2.5}$ mass and global DNA methylation.

Epigenetic effects following PM exposure have also been investigated using in vitro methods. Miousse et al. (2015) measured epigenetic changes at repetitive sequences and changes in methyltransferase gene expression in cultured murine macrophages (RAW264.7) after exposure to aqueous extracts of PM (number median aerodynamic diameter of 0.42 μm) collected from the lowest level of a multilevel underground parking deck at the University of Arkansas in Little Rock. Measurements of DNMT1 and DNMT3b mRNA transcripts following PM extract (50 μg/mL) exposure revealed a decrease after 24 hours compared to control ($p < 0.05$ and $p < 0.001$, respectively). No change was observed in the amount of DNMT3a mRNA measured at 24 hours, however, an increase was noted at 72 hours ($p < 0.001$). When the authors measured methyltransferase enzymatic activity, however, no change was observed after exposure to PM extracts. Several repetitive elements were studied to identify their methylation status and expression level after PM extract exposure. Weak hypomethylation of SINE B1/B2 was observed at the 24-hour time point control ($p < 0.01$ and $p < 0.05$, respectively). After 72 hours, methylation levels of SINE B1 returned to levels similar to that of the control; however, SINE B2 remained weakly hypomethylated ($p < 0.05$). Analysis of SINE B1/B2 expression did not reveal any differences between exposed and control cells at either time point. No change in methylation or expression of the other transposable element evaluated, L1, was observed.

In the same report, Miousse et al. (2015) also measured the change in methylation of major and minor satellites after 24 and 72 hours of exposure to aqueous extracts of PM. Only one change from the controls was observed. After 72 hours of exposure to 50 μg/mL PM extract, hypomethylation of the major satellites was reported. The authors again also measured the corresponding mRNA levels. No change in expression of either the major or minor satellites at either time point was observed.

10.2.3.2.2 Epidemiologic Evidence

Recent epidemiologic studies have expanded upon the examination of the relationship between $PM_{2.5}$ exposure and DNA methylation. These studies encompass both the examination of the methylation

of specific parts of the genome that may play an important role in carcinogenesis as well as an overall assessment of DNA methylation. Study characteristics, including $PM_{2.5}$ concentrations, study population, and approach to assigning $PM_{2.5}$ exposure, are detailed in Table 10-3.

Table 10-3 Study specific details and $PM_{2.5}$ concentrations from recent studies that examined DNA methylation.

Study Years	Location Population	Endpoints	Mean Concentration µg/m^3	Exposure assessment
†De Prins et al. (2013) (2010)	Flanders, Belgium (48 nonsmoking adults)	%5mdC	All-year: 17.1 Winter: 26.9 Summer: 15.2	Ambient concentration interpolated to 4 km grid cell by RIO as detailed in Janssen et al. (2008) and assigned to residential address
†Madrigano et al. (2011) (1999–2007)	Boston, MA (706 men, NAS)	%5mC of LINE-1 and Alu	28-day: 10.3 45-day: 10.3 60-day: 10.3 90-day: 10.4 180-day: 10.5	Ambient concentrations from one monitor
†Panni et al. (2016) (KORA F3: 2004–2005; KORA F4: 2006–2008; NAS: 1999–2007)	Germany (KORA F3: 500; KORA F4: 1,799; NAS: 657 white men)	% methylation for every CpG site	KORA F3: 20.0 KORA F4: 14.2 NAS: 10.6	Ambient concentrations from one monitor for each cohort
†Guo et al. (2014) (Jun–July 2008)	Beijing, China (Beijing Truck Driver Air Pollution Study, 60 truck drivers, 60 office workers)	%5mC of SATα, NBL2, and D4Z4	Truck drivers: 126.8 Office workers: 94.6	Average personal $PM_{2.5}$ on examination days using gravimetric samplers during 8 h of work
†Sanchez-Guerra et al. (2015) (Jun–July 2008)	Beijing, China (Beijing Truck Driver Air Pollution Study, 60 truck drivers, 60 office workers)	%5mC; %5hmC	Truck drivers: 126.8 Office workers: 94.6	Average personal $PM_{2.5}$ on examination days using gravimetric samplers during 8 h of work

Table 10-3 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies that examined DNA methylation.

Study Years	Location Population	Endpoints	Mean Concentration µg/m³	Exposure assessment
†Janssen et al. (2013) (2009–2012)	Limburg Province, Belgium (ENVIRONAGE; 240 mother-child pairs)	%5mdC	1–5 days: 16.9 6–12 days: 16.9 6–21 days: 16.7 22–28 days: 17.3 1st trimester: 16.7 2nd trimester: 17.4 3rd trimester: 18.2 Entire pregnancy: 17.4	Combination of kriging using land cover data from satellites and monitoring data at 4 km grid cells to estimate $PM_{2.5}$ at residential address as detailed in Janssen et al. (2008); temporal $R^2 > 0.80$, spatial $R^2 > 0.80$

%5mdC = percent 5-methyl-2'-deoxycytidine; %5mC = percentage of sum of methylated and unmethylated cytosine; %5hMC = percentage change in 5-hydroxymethylcytosine; Alu = short interspersed nucleotide element Alu; CpG = cytosine-guanine dinucleotide; ENVIRONAGE = environmental influence on early ageing; LINE-1 = long interspersed nucleotide element-1; NAS = Normative Aging Study.

†Studies published since the 2009 PM ISA.

Those studies that examined overall global methylation provide an assessment as to whether exposure to $PM_{2.5}$ can result in either hyper- or hypomethylation of DNA. De Prins et al. (2013) in a study conducted in Flanders, Belgium examined global DNA methylation (percentage 5-methyl-2′-deoxycytidine, %5 mdC) in 48 nonsmoking adults. The authors examined methylation at two-time periods, once in the summer and once in the winter, and whether any changes in methylation were associated with cumulative $PM_{2.5}$ exposures that were either short or long in duration (i.e., <1 week or up to a few months). In analyses combining the two sampling periods, De Prins et al. (2013) reported evidence indicating a reduction in overall DNA methylation across the lags examined with the magnitude of the reduction increasing over time, with the most pronounced reductions occurring at a 30-day lag (−0.14 [95% CI: −0.28, 0.00] for an IQR increase in $PM_{2.5}$ concentrations of 14.2 µg/m³) and 60-day lag (−0.18 [95% CI: −0.37, 0.01] for an IQR increase in $PM_{2.5}$ concentrations of 11.4 µg/m³). In seasonal analyses, there was also evidence of a reduction in methylation, but mostly in the summer and at shorter lags (i.e., 2-day and 3-day). In a subsequent genome-wide meta-analysis of DNA methylation in the Normative Aging Study (NAS) as well as the German KORA F3 and F4 studies, associations between $PM_{2.5}$ (trailing 2-day average), $PM_{2.5}$ (trailing 7-day average), and $PM_{2.5}$ (trailing 28-day average) was found to result in 1, 1, and 10 CpG sites that had changes in methylation, respectively (Panni et al., 2016). At the 10 CpG sites identified using 28-day average $PM_{2.5}$ exposure, 7 sites had higher methylation and 3 lower methylation. In a sensitivity analysis, the authors reported associations with $PM_{2.5}$ (trailing 28-day average) that were generally similar after adjustment for annual average $PM_{2.5}$ (trailing 1-year average). Although De Prins et al. (2013) and Panni et al. (2016) examined global DNA methylation across the entire genome, Madrigano et al. (2011) examined global methylation by focusing on specific portions of the genome, i.e., the LINE-1 and Alu repetitive elements. Within the NAS cohort, the authors examined multiple exposure time windows, 28, 45, 60, 90, and 180 days prior to biological sampling. For analyses

focusing on $PM_{2.5}$ exposure, Madrigano et al. (2011) reported some evidence of a small decrease in methylation at 45- and 60-days for only LINE-1, but 95% confidence intervals were large.

Additional studies that examined DNA methylation at specific sites of the genome relied on an assessment of $PM_{2.5}$ exposure using personal monitors. Guo et al. (2014) examined associations between personal $PM_{2.5}$ concentrations and blood DNA methylation (percentage 5 methylcytosine, %5 mC) of the tandem repeats SATα, NBL2, and D4Z4 in 60 office workers and 60 truck drivers within the Beijing Truck Driver Air Pollution Study. Biological samples from participants were provided twice, 1−2 weeks apart. The authors reported an inverse association between $PM_{2.5}$ concentrations and SATα methylation ($\beta = -1.35$, SE = 0.54) in office workers and truck drivers combined, with the association stronger in truck drivers ($\beta = -2.34$, SE = 0.94). There was also evidence of an inverse association between $PM_{2.5}$ concentrations and NBL2 methylation, but only in truck drivers ($\beta = -0.88$, SE = 0.84). These results indicate that higher exposures to $PM_{2.5}$ may result in the differential methylation of some parts of the genome. Sanchez-Guerra et al. (2015) also examined the Beijing Truck Driver Air Pollution Study cohort to examine methylation of both 5mC and 5-hydroxymethylcytosine (5hmC). Most DNA methylation studies focus on 5mC because it is often considered a marker of suppressed gene expression; however, 5mC is oxidized to 5hmC which is a potential marker of gene expression (Sanchez-Guerra et al., 2015). The authors examined whether PM exposure increases the oxidation of 5mC to 5hmC and subsequently increases blood levels of 5hmC. Using the same personal $PM_{2.5}$ measurements as the Beijing Truck Driver Air Pollution studies described previously, the authors did not report any evidence of an increase in 5hmC in response to $PM_{2.5}$ exposure.

Although the previous studies evaluated focused on DNA methylation in adults, a study conducted in Belgium examined the relationship between maternal $PM_{2.5}$ exposure and placental DNA methylation. Janssen et al. (2013) within the ENVIRONAGE cohort, examined the association between global DNA methylation and $PM_{2.5}$ exposure during each trimester of gestation and the entire pregnancy. The authors reported evidence of an overall reduction in placental DNA methylation by 2.2% (95% CI: −3.7, −0.73) when examining $PM_{2.5}$ exposures over the entire pregnancy. Analyses of individual trimesters as well as a model that simultaneously included each trimester provide evidence of the greatest reduction in methylation occurring in the 1st trimester, −2.4 and −2.1%, respectively.

10.2.3.3 Noncoding mRNAs

In addition to DNA methylation, interest in how environmental exposures affect miRNA expression has also increased since the 2009 PM ISA. miRNAs are small, evolutionary conserved, noncoding RNAs involved in the regulation of gene expression. Recently, animal toxicological studies have reported that exposure to various environmental stressors, including PM, can lead to alterations in miRNA expression and subsequent alterations in the expression of genetic information.

Borgie et al. (2015b) compared the effects of exposure to intact ambient $PM_{2.5}$ with aerodynamic diameters between 0.3 and 2.5 μm (described as $PM_{2.5-0.3}$) collected from an urban site in Beirut, Lebanon to that collected from a rural site in Byblos, Lebanon, which is located 35 km from Beirut. The authors measured miR-21, miR-26b, and miR-27a expression in cultured BEAS-2B cells. After exposure to PM collected from the urban location, miR-21 expression was increased compared to controls after exposure to both low and high concentrations (3 and 12 $\mu g/cm^2$). In contrast, PM collected from the rural location resulted in an increase compared to control for the high concentration exposure (12 $\mu g/cm^2$) only ($p < 0.05$), indicating that the $PM_{2.5}$ collected from the urban location may possess greater potency than that collected from the rural location.

10.2.3.4 Summary of Epigenetic Effects

Studies published since the completion of the 2009 PM ISA provide a broader evaluation of the relationship between $PM_{2.5}$ exposure and epigenetic effects. An animal toxicological study involving inhalation of $PM_{2.5}$ CAPs (Chicago) found methylation of the tumor suppressor gene p16 and upregulation of methylation enzymes in lung tissue (Soberanes et al., 2012). An in vitro experiment found similar results in the same study, as well as evidence for oxidative stress contributing to the effects. Other evidence from animal toxicological studies includes methylation of p16 and the repetitive line element LINE-1 in blood and lung tissue in association with $PM_{2.5}$ concentrations in a field study conducted in China (Ding et al., 2016) and upregulation of noncoding mRNA in an in vitro study involving $PM_{2.5}$ collected in Lebanon (Borgie et al., 2015b).

Recent epidemiologic studies of ambient and personal $PM_{2.5}$ concentrations generally reported some evidence of a change in DNA methylation. In studies examining both global methylation as well as methylation of specific genomic sites (i.e., CpG sites, LINE-1, Alu, SATα, and NBL2), there was evidence indicating hypomethylation in response to $PM_{2.5}$ exposure (Panni et al., 2016); Guo et al. (2014); (De Prins et al., 2013; Madrigano et al., 2011). However, there was also evidence of hypermethylation in some instances (Panni et al., 2016). A recent study in a cohort of mother-child pairs in Belgium also noted associations with $PM_{2.5}$ concentrations and changes in global DNA methylation (Janssen et al., 2013). Collectively, studies of $PM_{2.5}$ exposure and DNA methylation provide some evidence of epigenetic effects, but the broad number of biomarkers and measures of DNA methylation examined complicate the overall interpretation of results across studies.

10.2.4 Carcinogenic Potential

In the 2009 PM ISA (U.S. EPA, 2009), there were a small number of in vivo toxicological studies that examined carcinogenic potential. No evidence of increased tumor formation was found after chronic inhalation of diesel exhaust (Reed et al., 2004) or hardwood smoke (Reed et al., 2006) in a cancer-prone

mouse model. However, urban air in Brazil enhanced the formation of tumors in mice that were pretreated with urethane to initiate tumor formation (i.e., a model of tumor promotion) (Cury et al., 2000; Reymao et al., 1997). Because these in vivo studies evaluated effects of exposure to mixtures of PM and gases, they do not directly inform the current ISA, which identifies the hazard for effects after exposures to only the PM component of complex mixtures. Studies published since the 2009 PM ISA include an in vivo study of tumor promotion and an in vitro study of cell invasion, which is an indicator of metastasis.

Cangerana Pereira et al. (2011) exposed female Swiss mice to ambient $PM_{2.5}$ in downtown São Paulo, Brazil, 20 m from the roadside. Some animals were pretreated with the tumor initiator urethane, while others received saline. Exposed animals were housed in exposure chambers fitted with a filter designed to trap large particles but not $PM_{2.5}$. Control group animals were housed in exposure chambers fitted with a series of three filters designed to trap all ambient particles. After 60 days of exposure to 4.54 $\mu g/m^3$ and 17.66 $\mu g/m^3$ $PM_{2.5}$ in the filtered and nonfiltered chambers respectively, the authors counted the number of urethane-induced nodules (classified as adenomas) present at the pleural surface. The number of nodules observed in urethane-pretreated mice exposed to $PM_{2.5}$ was 4.0 ± 3.0; the number of nodules observed in the urethane-pretreated control group was 2.0 ± 2.0 ($p = 0.02$). Of animals treated with saline rather than urethane, neither those exposed to $PM_{2.5}$ nor those exposed to filtered air developed tumors. The results of this study, together with previously published observations that investigated the effect of air pollution on urethane-exposed mice (Cury et al., 2000; Reymao et al., 1997), demonstrate that ambient PM may have a promoting effect in lung carcinogenesis. The mechanism by which exposure to $PM_{2.5}$ enhanced tumorigenesis in this study was not explored; however, activation of inflammatory pathways, suppression of DNA repair, and an enhancement of DNA replication errors are all possibilities.

Yue et al. (2015) collected $PM_{2.5}$ over spring, summer, autumn, and winter from a peri-urban residential area of Taiyuan, China. Using A549 cells and $PM_{2.5}$ suspensions in a cell invasion assay, the authors report that cell invasion was greatest after exposure to $PM_{2.5}$ collected in the winter (p values not provided). The concentrations of 18 PM-bound PAHs were also measured. The authors reported that the amounts of PAHs measured for each season roughly corresponded to the extent to which cell invasion was observed for the same season, i.e., the amount of PM-bound PAH was greatest for that collected in the winter season, and the number of invading cells was greatest after exposure to PM collected during the winter season as well. When the authors repeated the experiment with a range of winter $PM_{2.5}$ suspension concentrations, the increase in invasive cells compared to controls was observed at the greatest doses only (3 $\mu g/mL$: $p < 0.05$; 10 $\mu g/mL$: $p < 0.01$). The authors also measured changes in mRNA of proteins important to the suppression and promotion of cell migration and invasion and noted a decrease in E-cad and TIMP-2 and an increase in Fib and MMP-2. Lastly, the authors also demonstrated the generation of ROS after exposure to the winter $PM_{2.5}$ with the DCFH-DA assay and demonstrated attenuation of cell migration in the presence of the antioxidant N-acetyl-L-cysteine, providing support for the contribution of ROS to additional events relevant to carcinogenesis.

In summary, although neither of the toxicological studies involving $PM_{2.5}$ exposure provides direct evidence of carcinogenesis, both demonstrated increased carcinogenic potential. Chronic inhalation of $PM_{2.5}$ CAPs collected in Brazil resulted in tumor promotion in an animal model. Furthermore, exposure to $PM_{2.5}$ in vitro increased cell invasion, a measure of metastatic potential, which correlated with PAH content. This effect was blocked by treatment with an antioxidant, suggesting a role for oxidative stress in mediating cell invasion. Epidemiologic studies provide initial evidence that exposure to long-term $PM_{2.5}$ concentrations may contribute to reduced cancer survival (see Section 10.2.5.3). This could involve an enhancement of tumor progression or metastasis/tissue invasion or some other mechanism.

10.2.5 Cancer Incidence, Mortality, and Survival

At the completion of the 2009 PM ISA, epidemiologic studies that examined the association between long-term $PM_{2.5}$ exposure and cancer primarily focused on lung cancer mortality, with a more limited number of studies examining lung cancer incidence and other types of cancers. Although these studies tended to support a relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality, the overall body of evidence was rather small and mostly limited to analyses and reanalyses of a few cohorts (i.e., American Cancer Society [ACS], Harvard Six Cities [HSC], Netherlands Cohort Study on Diet and Cancer [NLCS-Air], and Adventist Health and Smog Study [AHSMOG]). Since then, several new cohort studies and meta-analyses, as well as extensions and reanalyses of older cohorts, have examined $PM_{2.5}$ and both lung cancer incidence and mortality along with the potential relationship between long-term $PM_{2.5}$ exposure and cancers in other organs. Additionally, epidemiologic studies have examined the potential impact of $PM_{2.5}$ exposure on the survival of cancer patients. Overall, when evaluating recent epidemiologic studies, the strongest evidence demonstrating an association between long-term $PM_{2.5}$ exposure and cancer comes from studies that examine lung cancer incidence and mortality. This evidence is further supported by studies that examined associations in never smokers.

10.2.5.1 Lung Cancer

Epidemiologic studies that examine the relationship between long-term $PM_{2.5}$ exposure and lung cancer often focus on lung cancer mortality, which could be a reflection of the high case-fatality rate of lung cancer, resulting in measures of lung cancer mortality and incidence being comparable (Hamra et al., 2014). Recent studies of $PM_{2.5}$ and lung cancer have expanded upon the body of evidence for both lung cancer mortality and incidence. The following section focuses on those recent studies that adequately examine the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence using either modeled or monitored $PM_{2.5}$ concentrations. Many of the studies that examine lung cancer mortality are also evaluated in the long-term $PM_{2.5}$ exposure and mortality section (see Section 11.1.2). As a result, the focus of this section is specifically on issues inherent to the evaluation of the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality or incidence. Other studies with identified

limitations including, but not limited to, ecological study design, estimation of $PM_{2.5}$ concentrations for entire study duration from concentrations of other pollutants using conversion factors, and inadequate control for potential confounders are not the focus of this section. These studies are available at: https://hero.epa.gov/hero/particulate-matter.

Study characteristics including $PM_{2.5}$ concentrations, study population including number of deaths or cases, and exposure assignment approach for the large cohort studies that focused on national or regional analyses evaluated in the 2009 PM ISA, along with recent cohort studies that examine lung cancer mortality and incidence are detailed in Table 10-4. The results from these studies are highlighted in Figure 10-3, and provide evidence of generally consistent, positive associations across different exposure assignment approaches and study locations. Within the cohorts summarized in Table 10-4 and Figure 10-3, additional analyses were conducted to further examine the associations observed in the main analysis, which comprise the focus of the following sections.

Table 10-4 Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
Lung cancer mortality					
North America					
McDonnell et al. (2000)[a]	AHSMOG (California)	$PM_{2.5}$: 1973–1977 Follow-up: 1977–1992	Deaths: 13[e] Pop: 1,228[e]	31.9	Monthly average concentration for Airshed where participant resided
Laden et al. (2006)[b,c]	HSC Extension (Six U.S. cities)	$PM_{2.5}$: 1979–1987; 1985–1998[f] Follow-up: 1974–1998	Deaths: 226 Pop: 8,096	Across sites: 10.2–29.0 Overall mean: 16.4	One centrally located monitoring site in each city
Krewski et al. (2009)[b,d]	ACS-CPS II (1979–1983: 58 U.S. MSAs; 1999–2000: 116 U.S. MSAs)	$PM_{2.5}$: 1979–1983/ 1999–2000 Follow-up: 1982–2000	Deaths: NA Pop: 351,338 (1979–1983) 499,968 (1999–2000)	1979–1983: 21.2 1999–2000: 14.0	Average of all monitoring sites in each MSA

Table 10-4 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
†Jerrett et al. (2013)	ACS-CPS II (California)	$PM_{2.5}$: 1998–2002 Follow-up: 1982–2000	Deaths: 1,481 Pop: 73,711	14.1	LUR at geocoded addresses as detailed in Beckerman et al. (2013a) and van Donkelaar et al. (2010)
†Thurston et al. (2013)	ACS-CPS II (100 U.S. MSAs)	$PM_{2.5}$: 2000–2005 Follow-up: 1982–2004	Deaths: NA Pop: 445,860	14.2	Average of all monitoring sites in each MSA
†Turner et al. (2016)	ACS-CPS II	$PM_{2.5}$: 1999–2004 Follow-up: 1982–2004	Deaths: 16,432 Pop: 669,046	12.6	National-level hybrid LUR and BME interpolation model at geocoded address as detailed in Beckerman et al. (2013b); R^2 = 0.79
†Turner et al. (2011)	ACS-CPS II (1979–1983: 61 U.S. MSAs; 1999–2000: 117 U.S. MSAs; 1979–1983/ 1999–2000: 53 U.S. MSAs)	$PM_{2.5}$: (1) 1979–1983; (2) 1999–2000; (3) 1979–1983/ 1999–2000 Follow-up: 1982–2008	(1) Deaths: 772 Pop: 131,864 (2) Deaths: 1,042 Pop: 177,752 (3) Deaths: 714 Pop: 120,917	1979–1983: 21.1 1999–2000: 14.0 1979–1983/ 1999–2000: 17.6	Average of all monitoring sites in each MSA
†Turner et al. (2014)	ACS-CPS II	$PM_{2.5}$: 1999–2004 Follow-up: 1982–1988	Deaths: 1,921 Pop: 429,406	12.6	National-level hybrid LUR and BME interpolation model at geocoded address as detailed in Beckerman et al. (2013b); R^2 = 0.79
†Lipsett et al. (2011)	CTS (California)	$PM_{2.5}$: 1999–2005 Follow-up: 2000–2005	Deaths: 234 Pop: 73,489	15.6	IDW interpolation; limited to residences within 20 km from neighborhood and urban/regional monitors

Table 10-4 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
†Hart et al. (2011)	TrIPS (U.S.)	$PM_{2.5}$: 2000 Follow-up: 1985–2000	Deaths: 800 Pop: 53,814	14.1	Annual average concentration in year 2000 from nearest monitoring location to last known residential address
†Crouse et al. (2015)	CanCHEC (Canada)	$PM_{2.5}$: 1998–2006 Follow-up: 1991–2006	Deaths: 30,545 Pop, 2,521,525	8.9	10 km grid cells from three satellite instruments to residential postal code as detailed in van Donkelaar et al. (2014)
†Weichenthal et al. (2016)	CanCHEC (Ontario, Canada)	$PM_{2.5}$: 1998–2009 Follow-up: 1991–2009	Deaths: 3,200 Pop: 193,300	9.8	Mean concentration across all years of $PM_{2.5}$ data from provincial monitoring site within 5 km from residential address
†Pinault et al. (2016)	CCHS (Canada)	$PM_{2.5}$: 1998–2012 Follow-up: 2000–2011	Deaths: 2,700 Pop: 299,500	6.3	1 km grid cells from satellite measurements in combination with GEOS-Chem using geographically weighted regression to residential address as detailed in van Donkelaar et al. (2015)
†Lepeule et al. (2012)	HSC (U.S.)	$PM_{2.5}$: 1979–2009[g] Follow-up: 1974–2009	Deaths: 350 Pop: 8,096	Across sites: 11.4–23.6	One centrally located monitoring site in each city (1979–1988), average of all U.S. EPA monitors in each city (1986–2009)

Table 10-4 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
†Villeneuve et al. (2015)	CNBSS (Canada)	$PM_{2.5}$: 1998–2006 Follow-up: 1980–2005	Deaths: 1,011 Pop: 89,248	9.1[h]	10 km grid cells from three satellite instruments adjusted using GEOS-Chem to residential postal code as detailed in van Donkelaar et al. (2010) and van Donkelaar et al. (2014)
Europe					
Naess et al. (2007)[b]	Oslo Cohort (Oslo, Norway)	$PM_{2.5}$: 1992–1995 Follow-up: 1992–1998	Deaths: 1,453 Pop: 143,842	15.0	AirQUIS dispersion model
Brunekreef et al. (2009)l originally detailed in Beelen et al. (2008b)	NLCS-Air (Netherlands)	$PM_{2.5}$: 1987–1996 Follow-up: 1987–1996	Full cohort Deaths: 1,670 Case-Cohort deaths: 1,059 Pop: 117,528	28.2	Combination of IDW interpolation and land-use regression as detailed in Beelen et al. (2007)
†Carey et al. (2013)	National English (U.K.)	$PM_{2.5}$: 2002 Follow-up: 2003–2007	Deaths: 5,273 Pop: 830,842	12.9	1 km grid cells from air dispersion model based on estimation of emissions by sector; 1 km grid centroid linked to nearest residential postcode centroid as detailed in Atkinson et al. (2013); R^2 = 0.23–0.71
†Cesaroni et al. (2013)	RoLS (Rome, Italy)	$PM_{2.5}$: 2005 Follow-up: 2001–2010	Deaths: 12,208 Pop: 1,256,058	23.0	1 km grid Eulerian dispersion model to each residential address as detailed in Gariazzo et al. (2007) and Gariazzo et al. (2011)

Table 10-4 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
Asia					
†Wong et al. (2016)	(Hong Kong)	$PM_{2.5}$: 1998–2011 Follow-up: 1998–2011	Deaths: 1,408 Pop: 66,820	33.7	Combination of monitoring data, geospatial height information, and satellite data to estimate concentrations at geocoded residential address as detailed in Li et al. (2005) and Lai et al. (2010)
Lung cancer incidence					
North America					
†Puett et al. (2014)	NHS (U.S.)	$PM_{2.5}$: 1988–2007 Follow-up: 1994–2010	Cases: 2,155 Pop: 103,650	13.1[i]	GIS-based spatiotemporal model to each residential address as detailed in Yanosky et al. (2008); R^2 = 0.76–0.77
†Gharibvand et al. (2016)	AHSMOG-2 (U.S.)	$PM_{2.5}$: 2000–2001 Follow-up: 2002–2011	Cases: 250 Pop: 80,285	12.9	IDW interpolation to geocoded residential address
†Hystad et al. (2013)	NECSS (Canada)	$PM_{2.5}$: 1975–1994 Follow-up: 1994–1997	Cases: 2,390 Controls: 3,507	11.9	Spatiotemporal model to geocoded postal code of residential address as detailed in Hystad et al. (2012)
†Tomczak et al. (2016)	CNBSS (Canada)	$PM_{2.5}$: 1998–2006 Follow-up: 1980–2004	Cases: 932 Pop: 89,234	9.1[i]	10 km grid cells from three satellite instruments adjusted using GEOS-Chem to residential postal code as detailed in van Donkelaar et al. (2010)

Table 10-4 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies and studies evaluated in the 2009 PM ISA that examined lung cancer mortality and incidence.

Study	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m³	Exposure Assessment
Europe					
Brunekreef et al. (2009)[j] originally detailed in Beelen et al. (2008a)	NLCS-Air (Netherlands)	$PM_{2.5}$: 1987–1996 Follow-up: 1987–1996	Full cohort Cases: 1,940 Case-Cohort cases: 1,294 Pop: 111,816	28.3	Combination of IDW interpolation and land-use regression as detailed in Beelen et al. (2007)
†Raaschou-Nielsen et al. (2013)	ESCAPE (Europe)	$PM_{2.5}$: 2008–2011 Follow-up: 1990s[k]	Cases: 2,095 Pop: 312,944	Across sites: 6.6–31.0	LUR at geocoded addresses as detailed in Eeftens et al. (2012a)
†Raaschou-Nielsen et al. (2016)	TRANSPHORM (Europe)	$PM_{2.5}$: 2008–2011 Follow-up: 1990s[l]	Cases: 1,878 Pop: 245,782	Across sites: 6.6–31.0	LUR at geocoded addresses as detailed in Eeftens et al. (2012a)
†Hart et al. (2015)	NLCS-Air (Netherlands)	$PM_{2.5}$: 1987–1996 Follow-up: 1986–2003	Cases: 3,355 Pop: 120,852	28.3	Combination of IDW interpolation and land-use regression as detailed in Beelen et al. (2007) and Beelen et al. (2008a)

ACS-CPS = American Cancer Society-Cancer Prevention Study; AHSMOG = Adventist Health Study on Smog; BME = Bayesian maximum entropy; CanCHEC = Canadian Census Health and Environment Cohort; CCHS = Canadian Community Health Survey; CNBSS = Canadian National Breast Screening Study; CTS = California Teacher's Study; ESCAPE = European Study of Cohorts for Air Pollution Effects; GIS = Geographic Information System; HSC = Harvard six cities cohort; IDW = Inverse distance-weighted; NECSS = National Enhanced Cancer Surveillance System project; NHS = Nurses' Health Study; NCLS-Air = Netherlands Cohort Study on Diet and Cancer; RoLS = Rome Longitudinal Study; TrIPS = Trucking Industry Particle Study; TRANSPHORM = European Study of Transport-related Air Pollution and Health Impacts-Integrated Methodologies for Assessing Particulate Matter.

[a]Evaluated in 2004 PM AQCD.

[b]Evaluated in 2009 PM ISA.

[c]Builds off the studies conducted by Dockery et al. (1993) and Krewski et al. (2000).

[d]Builds off the studies conducted by Pope et al. (1995) and Pope et al. (2002).

[e]Males only.

[f]During this period $PM_{2.5}$ estimated using city-specific regression equations based on extinction coefficient.

[g]For a subset of years when $PM_{2.5}$ was not monitored 1986–1988 through 1998, $PM_{2.5}$ concentrations were estimated from PM_{10}.

[h]Median concentration.

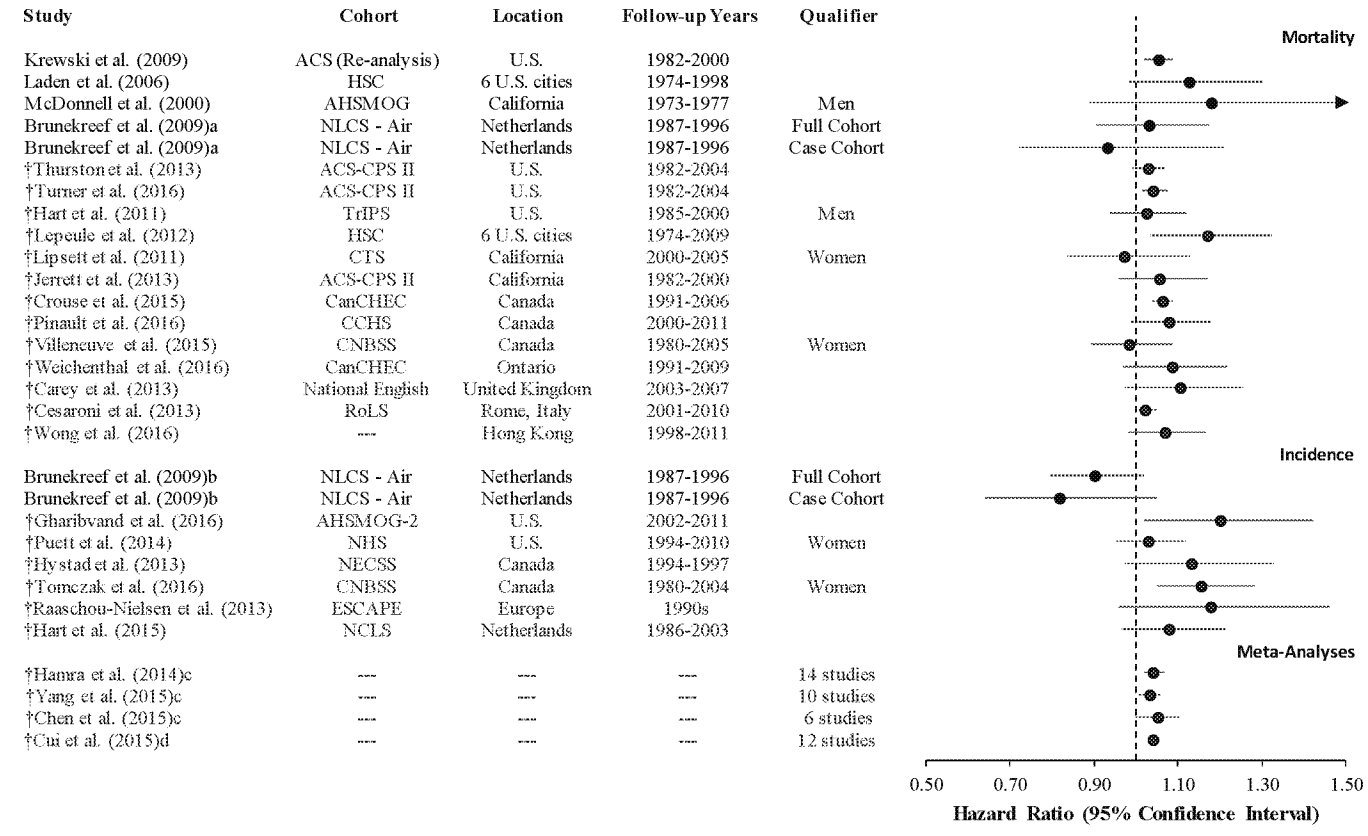
[i]Overall 72 mo cumulative average $PM_{2.5}$ concentration.

[j]$PM_{2.5}$ exposure assigned to residential address at 1986, study only reports population for all natural causes, not lung cancer, in Case-Cohort, and Beelen et al. (2008b) and Beelen et al. (2008a) presented the results of Brunekreef et al. (2009) prior to its publication.

[k]Only 14 or the 17 cohorts were examined for lung cancer, of the cohorts examined initial recruitment started generally in the 1990s with an average follow-up time of 12.8 years.

[l]TRANSPHORM used 14 of the 17 cohorts in the ESCAPE study where initial recruitment started generally in the 1990s with an average follow-up time of 13.1 years.

†Studies published since the 2009 PM ISA.



ACS-CPS = American Cancer Society-Cancer Prevention Study; AHSMOG = Adventist Health Study on Smog; CanCHEC = Canadian Census Health and Environment Cohort; CCHS = Canadian Community Health Survey; CNBSS = Canadian National Breast Screening Study; CTS = California Teacher's Study; ESCAPE = European Study of Cohorts for Air Pollution Effects; HSC = Harvard six cities cohort; NECSS = National Enhanced Cancer Surveillance System project; RoLS = Rome Longitudinal Study; TrIPS = Trucking Industry Particle Study. Hazard ratios are standardized to a 5 $\mu g/m^3$ increase in annual $PM_{2.5}$ concentrations.

[a]Lung cancer mortality results originally reported in Beelen et al. (2008b).

[b]Lung cancer incidence results originally reported in Beelen et al. (2008a).

[c]Risk estimate is a combination of lung cancer mortality and incidence estimates.

[d]Risk estimate is only for lung cancer mortality.

Corresponding quantitative results are reported in Supplemental Material. See U.S. EPA (2018).

Note: †Studies published since the 2009 PM ISA. Studies in black were included in the 2009 PM ISA.

Figure 10-3 Summary of associations reported in previous and recent cohort studies that examined long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence.

10.2.5.1.1 Lung Cancer Mortality

Recent studies that examined the association between long-term $PM_{2.5}$ exposure and lung cancer mortality have attempted to account for the potential confounding effects of exposure to cigarette smoke

through detailed information on smoking status as well as exposure to second-hand smoke (SHS). These studies have assessed the role of smoking status on the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality through two approaches, either including smoking status as a covariate in the main statistical model or examining whether smoking status modifies the $PM_{2.5}$-lung cancer mortality association. The following section discusses both approaches, focusing first on those studies that had individual-level data on smoking status and then those studies that used proxy measures to account for smoking status within the study population.

Individual-Level Data on Smoking Status

The majority of studies that examined the $PM_{2.5}$-lung cancer mortality relationship focused on the ACS-CPS II cohort, building off the initial work presented in Pope et al. (1995) and then reanalyzed in subsequent studies (e.g., (Krewski et al., 2009)). These studies differed primarily in the years of $PM_{2.5}$ data examined, years of follow-up, exposure assignment approaches, and geographic extent of the cohort examined (i.e., national or specific location; Table 10-4). A summary of the results from studies that focused on the ACS-CPS II cohort that are evaluated in this section are detailed in Table 10-5.

Whereas the initial ACS-CPS II studies focused on assigning exposure using the average $PM_{2.5}$ concentrations across all monitors, Jerrett et al. (2013) conducted a more detailed exposure assessment using LUR in a subset of the full cohort limited to California. The authors reported a positive association with lung cancer mortality (HR = 1.06 [95% CI: 0.96, 1.17]). Although specific to California, the results of Jerrett et al. (2013) are consistent with those observed in the full cohort using cruder exposure assessment techniques, which includes Krewski et al. (2009) as well as a recent analysis by Thurston et al. (2013) that focused on mortality and long-term exposure to $PM_{2.5}$ components and sources. Using a similar exposure assignment approach as Krewski et al. (2009), Thurston et al. (2013) reported a HR = 1.03 (95% CI: 0.99, 1.08) for lung cancer mortality in a model adjusting for a range of individual- and ecological-level covariates including cigarette smoking history.

Table 10-5 Summary of results from studies that examined long-term $PM_{2.5}$ exposure and mortality in the American Cancer Society-Cancer Prevention Study II.

Study	ACS-CPS II Population	Location	Result[a]
Krewski et al. (2009)	Full cohort	National	1.05 (1.02, 1.09)
†Jerrett et al. (2013)	Full cohort	California	1.06 (0.96, 1.17)
†Thurston et al. (2013)	Full cohort	National	1.03 (0.99, 1.08)
†Turner et al. (2016)	Never smokers	National	1.04 (1.01, 1.08)
†Turner et al. (2011)	Full cohort	National	1979–1983: 1.07 (0.99, 1.16) 1999–2000: 1.13 (1.01, 1.25) 1979–1983; 1999–2000: 1.09 (0.98, 1.21)
†Turner et al. (2014)	Full cohort[b]	National	Never smoker (high vs. low): 1.26 (0.90, 1.77) Current smoker (high vs. low): 1.19 (1.03, 1.38)

[a]All results are for a 5 $\mu g/m^3$ increase in $PM_{2.5}$ concentrations except Turner et al. (2014) where results were based on comparing results between the 25th percentile (≤10.59 $\mu g/m^3$) and 75th percentile (>14.44 $\mu g/m^3$) of $PM_{2.5}$ concentrations.

[b]Study population that produced these results was smaller than the total population of the study detailed in Table 10-4, Never Smokers (Lung Cancer Deaths = 144, Population = 149,617); Current Smokers (Lung Cancer Deaths = 793, Population = 65,275).

†Studies published since the 2009 PM ISA.

Using a more refined exposure assignment approach in the full ACS-CPS II cohort, Turner et al. (2016) examined associations between both overall $PM_{2.5}$ concentrations using a national-level hybrid LUR Bayesian maximum entropy interpolation (LURBME) model as well as $PM_{2.5}$ concentrations decomposed into near-source (LUR) and regional (LURBME-LUR) components. The authors reported a positive association between overall $PM_{2.5}$ from the LURBME model and lung cancer mortality (HR = 1.04 [95% CI 1.01, 1.08]). Positive associations were also observed when examining both the near-source (HR = 1.08 [95% CI: 0.98, 1.18]) and regional (HR = 1.04 [95% CI: 1.00, 1.07]) components of ambient $PM_{2.5}$ concentrations. The results of Turner et al. (2016) provide evidence that within the ACS-CPS II, regardless of the exposure assignment approach used there is evidence of a consistent positive association between long-term $PM_{2.5}$ exposure and lung cancer mortality (see Figure 10-3).

As detailed above, traditionally ACS-CPS II studies have included covariates for smoking status or exposure to SHS in statistical models, but have not accounted for potential residual confounding by cigarette smoke. Often the examination of the association between long-term air pollution exposure, including $PM_{2.5}$, and lung cancer mortality in never smokers has been limited by the small number of lung cancer deaths (Turner et al., 2011). Within the ACS-CPS II cohort Turner et al. (2011) examined lung cancer mortality only in never smokers by using the three $PM_{2.5}$ exposure periods (i.e., 1979–1983,

1999–2000, and average of 1979–1983 and 1999–2000) initially detailed in Pope et al. (2002). Across the three different exposure periods and the three different statistical models examined, which varied by the degree of individual- and ecological covariates included, associations were consistently positive with HRs ranging from 1.07–1.14. In the fully adjusted model, which in addition to controlling for a number of individual-level covariates also controlled for county-level residential radon concentrations, Turner et al. (2011) found little evidence that radon confounded the $PM_{2.5}$-lung cancer mortality relationship, reporting a HR = 1.07 (95% CI: 0.99, 1.16) and HR = 1.13 (95% CI: 1.10, 1.25) for 1979–1983 and 1999–2000, respectively.

In Turner et al. (2011) the examination of the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality was on never smokers, while Turner et al. (2014) took this initial analysis one step further and focused on whether there is evidence of an interaction between long-term $PM_{2.5}$ exposure and smoking status. While the discussion of the interaction between smoking status and $PM_{2.5}$ is more informative in identifying populations potentially at increased risk of a PM-related health effect (see Chapter 12), analyses focusing solely on never smokers and current smokers in Turner et al. (2014) provide additional supporting evidence for a relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality. In analyses comparing lung cancer mortality in never smokers exposed to low (≤25th percentile = 10.59 $\mu g/m^3$) and high (>75th percentile = 14.44 $\mu g/m^3$) $PM_{2.5}$ concentrations the authors reported a HR = 1.26 (95% CI: 0.90, 1.77) while for current smokers the authors reported a HR = 1.19 (95% CI: 1.03, 1.38). Although 95% confidence intervals are larger for the strata of never smokers due to the small number of cases, the results of Turner et al. (2014) support a relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality, particularly in locations with higher $PM_{2.5}$ concentrations.

Similar to the ACS-CPS II cohort, the HSC cohort had detailed individual-level data on smoking status. Lepeule et al. (2012) extended the analysis of the original HSC cohort and reported a positive association between $PM_{2.5}$ concentrations in the 1–3 years prior to lung cancer death (or censoring; HR = 1.17 [95% CI: 1.03, 1.32]). This lag structure between $PM_{2.5}$ exposure and lung cancer mortality was also observed in the Canadian Community Health Survey (CCHS) cohort. In models controlling for smoking status using individual-level data, Pinault et al. (2016) reported a HR = 1.08 (95% CI: 0.99, 1.18) when examining $PM_{2.5}$ exposures over the 3 years prior to death.

In additional analyses stratifying by smoking status, Lepeule et al. (2012) reported that the association between $PM_{2.5}$ and lung cancer mortality persisted in never smokers, but the 95% confidence intervals were large (HR = 1.12 [95% CI: 0.73, 1.70]) due to only 26 out of the 350 lung cancer deaths occurring in never smokers. Overall, the association largest in magnitude for $PM_{2.5}$ and lung cancer mortality were observed for former smokers (HR = 1.40 [95% CI: 1.14, 1.73]). The results of Lepeule et al. (2012) indicating an association larger in magnitude for never smokers compared to the full cohort are consistent with the results of Carey et al. (2013) in a National English cohort. Carey et al. (2013) reported a HR = 1.22 (95% CI: 1.08, 1.41) in a model that included covariates for smoking and BMI. In models

including additional variables for education and income separately the lung cancer mortality association was attenuated, but remained positive (with income: HR = 1.05; with education HR = 1.11). When restricting the analysis to never smokers, the authors observed a rather large increase in the lung cancer mortality association (HR = 1.41 [95% CI: 1.22, 1.62]).

There was no evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer mortality in two cohorts of women, the California Teachers Study (CTS) and the Canadian National Breast Screening Survey (CNBSS). In the CTS, 67% of participants were never smokers, and Lipsett et al. (2011) reported no evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer mortality (HR = 0.97 [95% CI: 0.84, 1.13]). The results from the CTS cohort are consistent with the CNBSS cohort, which had a lower percentage of never smokers, 49.3% (HR = 0.98 [95% CI: 0.89, 1.09]) (Villeneuve et al., 2015). In the CTS cohort, the null $PM_{2.5}$-lung cancer mortality association persisted in several sensitivity analyses including, but not limited to, only post-menopausal women as well as women who did not relocate during follow-up. However, when focusing on only never smokers, Lipsett et al. (2011) reported that the association between long-term $PM_{2.5}$ exposure and lung cancer mortality was positive, but imprecise (HR = 1.27 [95% CI: 0.91, 1.78]) due to the small number of lung cancer deaths (i.e., 50) in this subset of the cohort, which is consistent with never smoker analyses in both Lepeule et al. (2012) and Carey et al. (2013). Villeneuve et al. (2015) in the CNBSS cohort only reported results by smoking status in analyses of all cancers, and did not observe a similar pattern of associations as the other cohorts when stratifying by smoking status (i.e., associations larger in magnitude for never smokers).

Across the lung cancer mortality studies, the magnitude of the association was generally consistent in areas where mean $PM_{2.5}$ concentrations were generally below 15 $\mu g/m^3$ (i.e., in the U.S. and Canadian cohorts), and below 30 $\mu g/m^3$ in all studies except Wong et al. (2016) (Table 10-4). Wong et al. (2016) in a study conducted in Hong Kong that examined long-term $PM_{2.5}$ exposure and all cancers, in a model controlling for smoking status, reported an association for lung cancer mortality similar in magnitude (HR = 1.07 [95% CI: 0.98, 1.17]) to that observed in the other cohort studies. Additionally, unlike the other studies evaluated in this section where the age of study participants was broader, the cohort was limited to those 65 years of age and older. The interpretation of these results is complicated when examining associations by smoking status. For men, 85% of the lung cancer mortality cases were in ever smokers, while for women 72% were in never smokers. However, when examining associations in each subset of the cohort, no evidence of an association was observed in women that were never smokers or ever smokers, while the strongest association was in ever smoker men (HR = 1.17 [95% CI: 1.02, 1.33]). There was evidence of a positive association for never smoker men, but the 95% confidence intervals were large due to the small number of cases (HR = 1.09 [95% CI: 0.72, 1.66]).

Proxy Measures of Smoking Status

In addition to the cohorts discussed above that controlled for smoking status or examined whether there was evidence of effect measure modification by smoking status, several cohorts examined the

association between long-term $PM_{2.5}$ exposure and lung cancer mortality without the ability to account for smoking status through detailed individual-level data. In an analysis of the Canadian Census Health and Environment Cohort (CanCHEC), Crouse et al. (2015) using a 7-year moving window of $PM_{2.5}$ concentrations for each year of follow-up reported a HR = 1.03 (95% CI: 1.01, 1.05). To adjust for smoking status and obesity, the authors used ancillary data on smoking and obesity to adjust for both risk factors not included in the original data set. Applying this method to account for smoking status and obesity resulted in a slightly larger HR = 1.08 (95% CI: 1.04, 1.09). A subsequent analysis of CanCHEC conducted by Weichenthal et al. (2016) limited to Ontario and focusing on $PM_{2.5}$ oxidative potential (see Section 10.2.5) also reported results for $PM_{2.5}$ and they were larger in magnitude (HR = 1.12 [95% CI: 1.00, 1.25]) compared to those observed in the full CanCHEC study (Crouse et al., 2015). The difference in results between the Ontario and national CanCHEC studies could be attributed to several factors (e.g., demographic differences), along with the exposure assignment approach employed in each study (see Table 10-4). Similar to Crouse et al. (2015), Weichenthal et al. (2016) indirectly adjusted for smoking status and obesity, by including a variable in the statistical model that accounted for both through examination of a secondary nationally representative data set (i.e., CCHS), and found the results were relatively similar to that observed in the main model (HR = 1.09 [95% CI: 0.97, 1.22]). Cesaroni et al. (2013) in the Rome Longitudinal Study (RoLS) also used proxy measures to account for smoking status, but relied on measures of neighborhood socioeconomic level and pre-existing comorbidities, which have been shown to be associated with smoking, to develop an indicator variable meant to control for smoking status. Using time-dependent annual $PM_{2.5}$ concentrations the authors reported a positive association (HR = 1.02 [95% CI: 1.00, 1.05]) between $PM_{2.5}$ exposure and lung cancer mortality.

While the previous studies detailed within this section focused specifically on ambient $PM_{2.5}$ exposure and lung cancer mortality in the general population, Hart et al. (2011) examined ambient air pollution exposures and cause-specific mortality, including lung cancer, in an occupational cohort from the Trucking Industry Particle Study (TrIPS). The TrIPS cohort consisted of men employed in the trucking industry, and similar to the CanCHEC and RoLS cohorts the authors did not have individual-level data to account for smoking status. However, unlike the CanCHEC and RoLS cohorts the authors did not attempt to indirectly adjust for smoking status. While most of the studies detailed in this section relied on multiple years of $PM_{2.5}$ data, only data from the year 2000 was available. In analyses focusing on the full cohort, the authors reported a positive association between $PM_{2.5}$ exposure and lung cancer mortality (HR = 1.03 [95% CI: 0.94, 1.12]). To further assess the association between $PM_{2.5}$ exposure and cause-specific mortality, Hart et al. (2011) conducted a sensitivity analysis that excluded long haul truckers, which potentially reduces exposure misclassification by focusing on those truckers that return home nightly due to $PM_{2.5}$ exposures being assigned at the residential address. In the subset analysis, the authors tended to observe associations larger in magnitude across mortality outcomes compared to the full cohort although confidence intervals were larger (lung cancer; HR = 1.08 [95% CI: 0.97, 1.21]).

Summary

In summary, results from recent epidemiologic studies that examined the association between long-term $PM_{2.5}$ exposure and lung cancer mortality are generally consistent with those studies evaluated in the 2009 PM ISA (Figure 10-3). Additional reanalyses of the ACS cohort using different years of $PM_{2.5}$ data and follow-up along with exposure assignment approaches and geographic extent of the cohort continue to provide evidence of consistent positive associations between long-term $PM_{2.5}$ exposure and lung cancer mortality. Additional epidemiologic studies that used individual-level data to control for smoking status conducted both within the U.S. and internationally, also provide evidence of generally consistent positive associations. The positive associations observed across studies are further supported by studies that conducted analyses focusing on never smokers that also reported positive associations, albeit with wide confidence intervals due to the small number of lung cancer mortality cases within the population of never smokers. There was no evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer mortality in two cohorts of women (i.e., CTS and CNBSS cohorts). However, an analysis of never smokers in the CTS cohort reported evidence of a positive association that was consistent with the other studies evaluated within the section that conducted analyses of never smokers. The results across studies that had individual-level data on smoking status are supported by additional epidemiologic studies in cohorts that relied upon proxy measures to account for smoking status.

10.2.5.1.2 Lung Cancer Incidence

Although there is a high case-fatality rate for lung cancer, at the completion of the 2009 PM ISA (U.S. EPA, 2009), an uncertainty identified was the limited number of studies that examined lung cancer incidence. These studies did not provide evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer incidence. Since the completion of the 2009 PM ISA, a larger number of studies have examined lung cancer incidence, but overall the total number of studies remains small compared to lung cancer mortality. Similar to some of the lung cancer mortality studies, the lung cancer incidence studies also conducted stratified analyses by smoking status, which can contribute to assessing whether a relationship exists between long-term $PM_{2.5}$ exposure and lung cancer by focusing on never smokers. A unique feature of lung cancer incidence studies that also allows for further assessment of the $PM_{2.5}$-lung cancer relationship is their ability to examine associations by the histological subtype of lung cancer. Specifically, an assessment of adenocarcinoma, the only subtype that develops in nonsmokers, can contribute to further accounting for residual confounding due to smoking (Hystad et al., 2013). The following lung cancer incidence studies examine both associations stratified by smoking status, and in most cases also histological subtype.

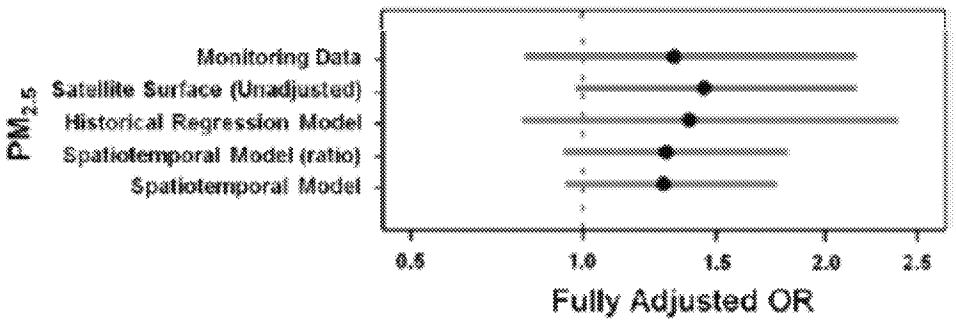
Within the U.S., the Nurses' Health Study (NHS) cohort (Puett et al., 2014) and the AHSMOG-2 cohort (Gharibvand et al., 2016) both examined the association between long-term $PM_{2.5}$ exposure and lung cancer incidence. In the NHS cohort, Puett et al. (2014) used 72-month average predicted $PM_{2.5}$ concentrations as the exposure metric, but due to the lack of $PM_{2.5}$ monitors prior to 1999, $PM_{2.5}$

concentrations for earlier time periods of the study were estimated from PM_{10}. The authors reported evidence of a small positive association with wide confidence interval for lung cancer incidence in the full cohort when adjusting for smoking status and SHS exposure (HR = 1.03 [95% CI: 0.95, 1.12] when examining 72-month average $PM_{2.5}$ concentrations). In a subset analysis of only never smokers the authors reported an association larger in magnitude (HR = 1.12 [95% CI: 0.87, 1.44]), which was also observed when combining never smokers and former smokers that had quit more than 10 years ago (HR = 1.17 [95% CI: 1.03, 1.33]). There was no evidence of an association when examining the combination of current smokers and former smokers that stopped smoking within the last 10 years. Lung cancer incidence was further evaluated through an examination of histological subtypes, specifically adenocarcinomas which comprise 44% of all lung cancer cases (Puett et al., 2014). Compared to the full cohort, when examining adenocarcinomas, the authors observed associations larger in magnitude for both the full cohort and the subset of never smokers and former smokers that had quit more than 10 years ago with HRs ranging from 1.15−1.29, but across categories confidence intervals were wide.

Gharibvand et al. (2016) within the AHSMOG-2 cohort examined mean monthly $PM_{2.5}$ concentrations over a 24-month period. In the cohort approximately 80% of the participants were never smokers, and they represented 46% of the lung cancer cases. In the full cohort, Gharibvand et al. (2016) reported evidence of a positive association when examining monthly average $PM_{2.5}$ concentrations (HR = 1.20 [95% CI: 1.02, 1.42]), which was similar in magnitude when examining both never (HR = 1.15 [95% CI: 0.95, 1.39]) and ever (HR = 1.22 [95% CI: 1.01, 1.48]) smokers. Overall, the lung cancer incidence associations in the AHSMOG-2 cohort are larger in magnitude than those observed in Puett et al. (2014), which could be attributed to the larger percentage of never smokers or long-term former smokers in the study population. On average, within the cohort, ever smokers quit smoking 24 years ago (Gharibvand et al., 2016). In an attempt to assess the influence of differences in time-activity on the observed associations, the authors examined average daily time spent outdoors and time lived at each residential location and found in both instances associations were similar in magnitude to the full cohort for those people that spent more than 1 hour per day outdoors and resided at their current address for more than 5 years. Of the lung cancer cases, approximately 66% were adenocarcinomas, which is a much larger percent than was observed in the NHS cohort, but the authors did not examine associations by histological subtype.

Additional national cohorts conducted in Canada, provide evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer incidence that is similar in magnitude to that observed in AHSMOG-2 (Gharibvand et al., 2016). Hystad et al. (2013) used a case-control study with participants identified through the National Enhanced Cancer Surveillance System (NECSS) project. To reduce exposure misclassification and account for time-activity, the study was limited to cases and controls that had complete 20-year residential histories. In fully adjusted models that accounted for smoking status, the authors reported evidence of a positive association between annual $PM_{2.5}$ concentrations and lung cancer incidence (OR = 1.14 [95% CI: 0.97, 1.33]). Hystad et al. (2013) further assessed whether the exposure assignment approach used influenced the $PM_{2.5}$-lung cancer incidence association observed, and found

that across exposure assignment approaches which included using fixed-site monitoring data, satellite data, a historical regression model, and two different versions of a spatiotemporal model, the magnitude of associations was generally consistent (Figure 10-4). In additional analyses stratified by smoking status, the authors observed the strongest association among former smokers (OR = 1.20 [95% CI: 0.98, 1.48]), with no evidence of an association in never smokers (0.97 [95% CI: 0.62, 1.53]), which could be attributed to only 6% of all lung cancer cases in this population being never smokers. In histological subtype analyses, Hystad et al. (2013) did not observe a clear relationship between long-term $PM_{2.5}$ exposure and one subtype, which differs from the results of Puett et al. (2014), which indicated associations larger in magnitude for adenocarcinomas.



Source: Permission pending, Hystad et al. (2013).

Figure 10-4 $PM_{2.5}$—lung cancer incidence odds ratios (OR) for a 10 µg/m^3 increase in $PM_{2.5}$ concentrations from sensitivity analyses using different exposure assignment approaches in the Canadian National Enhanced Cancer Surveillance System (NECSS) project.

The main results of Hystad et al. (2013) are consistent with those observed in another Canadian cohort (CNBSS) by Tomczak et al. (2016), which is the same cohort that was examined for lung cancer mortality by Villeneuve et al. (2015) detailed above. In a model controlling for smoking status and other SES-related variables, the authors observed evidence of an increase in lung cancer incidence in this cohort of women (HR = 1.16 [95% CI: 1.05, 1.28]). In analyses stratified by smoking status, no association was observed for never smokers, while the association for ever smokers was consistent with that observed in the full cohort, indicating that this subset of the cohort is responsible for the overall association (HR = 1.18 [95% CI: 1.06, 1.32]). Tomczak et al. (2016) also conducted histological subtype analyses and observed evidence of a positive association for small cell carcinoma and adenocarcinoma. Although the 95% confidence intervals for the histological subtype analyses in Hystad et al. (2013) were large resulting in the inability to clearly identify differences across subtypes, the central estimates were also largest in magnitude for small cell carcinoma and adenocarcinoma.

The examination of $PM_{2.5}$ and lung cancer incidence in the European Study of Cohorts for Air Pollution Effects (ESCAPE) study resulted in an association similar in magnitude to that observed in the AHSMOG-2, NECSS, and CNBSS cohorts discussed above (HR = 1.18 [95% CI: 0.96, 1.46]) (Raaschou-Nielsen et al., 2013). The results of Raaschou-Nielsen et al. (2013) are the same as those reported by Raaschou-Nielsen et al. (2016) as part of the European Study of Transport-related Air Pollution and Health Impacts-Integrated Methodologies for Assessing Particulate Matter (TRANSPHORM) project, which also used data from the ESCAPE study, but focused on associations between long-term $PM_{2.5}$ component exposures and lung cancer incidence. In additional analyses conducted by Raaschou-Nielsen et al. (2013) that attempted to reduce the impact of exposure misclassification by focusing on those residents who did not change residence during the follow-up period, the authors reported an association similar in magnitude to the full cohort (HR = 1.20 [95% CI: 0.96, 1.51]), which is consistent with the analysis focusing on people that resided at their residential location for over 5 years conducted by Gharibvand et al. (2016) in the AHSMOG-2 cohort. Analyses stratified by smoking status did not provide strong evidence for differences among never, former, and current smokers, but associations were largest in magnitude for never (HR = 1.21) and former (HR = 1.41) smokers although 95% confidence intervals were large. When examining histological subtypes, Raaschou-Nielsen et al. (2013) observed a positive association for only adenocarcinomas (HR = 1.51 [95% CI: 1.10, 2.08]).

In another study conducted in Europe, Hart et al. (2015), in a cohort in the Netherlands (NLCS-Air), also observed evidence of a positive association between long-term $PM_{2.5}$ exposure and lung cancer incidence in models that included a variable to adjust for smoking status (HR = 1.08 [95% CI: 0.96, 1.21] for 1987−1996). Within this study a case-cohort approach was used as detailed in the original NCLS-Air cohort (Brunekreef et al., 2009; Beelen et al., 2008a). Interestingly the results of Hart et al. (2015) differ from those observed in the original NCLS-Air cohort analysis where no evidence of an association was reported with lung cancer incidence (Figure 10-3). Although not explicitly detailed in Hart et al. (2015) there are differences with the original NLCS-Air studies that could contribute to the disparate results observed between the original and extended analyses, specifically (1) an additional 6 years of follow-up, (2) the transition of some individuals to being classified as cases, (3) the exclusion of individuals without exposure or smoking status information, and (4) the use of age in years as the timescale instead of time in study (Hart, 2017b). In addition to providing overall results, Hart et al. (2015) also attempted to adjust the observed association to account for exposure measurement error by using information from a validation study involving personal and near-home outdoor measurements of 47 nonsmokers from 2004−2005. After adjusting for exposure measurement error using a regression calibration analysis the $PM_{2.5}$-lung cancer incidence association increased in magnitude, but had larger confidence intervals (1.17 [95% CI: 0.93, 1.47]). The approach by Hart et al. (2015) along with those less computationally intensive approaches detailed in Raaschou-Nielsen et al. (2013) in the ESCAPE study and Gharibvand et al. (2016) in the AHSMOG-2 cohort consistently demonstrate that $PM_{2.5}$-lung cancer incidence associations are robust when trying to account for or reduce the potential impact of exposure measurement error. However, it should be noted that in Hart et al. (2015) residential address information was only available at baseline and the validation study was conducted after the follow-up period ended,

both of which contribute some level of uncertainty in adjusting the association to account for exposure measurement error. Hart et al. (2015) also conducted histological subtype analyses, and observed positive associations across all subtypes, but no clear difference in associations between subtypes existed.

Summary

Recent epidemiologic studies build upon the limited number of studies evaluated in the 2009 PM ISA that examined the association between long-term $PM_{2.5}$ exposure and lung cancer incidence, and provide evidence of consistent positive associations (Figure 10-3). Consistent with lung cancer mortality studies, studies that conducted analyses focusing on the subset of the cohort that were never smokers generally reported evidence of positive associations, albeit with wide confidence intervals due to the small number of never smokers within the cohorts. A subset of the studies focusing on lung cancer incidence also examined histological subtype, which provided some evidence of positive associations for adenocarcinomas, the only subtype of lung cancer observed in never smokers. However, in some studies the examination of associations by histological subtype were limited due to the small number of never smokers included within the cohort (e.g., NECSS cohort). In several studies, the $PM_{2.5}$-lung cancer incidence associations observed were further evaluated in sensitivity analyses that attempted to reduce exposure measurement error by accounting for length of time at residential address, examining different exposure assignment approaches, and conducting regression calibration to account for exposure measurement error. Across all approaches, associations between long-term $PM_{2.5}$ exposure and lung cancer incidence were found to remain relatively unchanged, but in some cases confidence intervals increased in width.

10.2.5.1.3 Copollutant Models

Across the epidemiologic studies that examined associations between long-term $PM_{2.5}$ exposure and lung cancer incidence and mortality, only a few examined potential copollutant confounding. Jerrett et al. (2013) in the ACS-CPS II cohort conducted copollutant analyses with NO_2 and O_3. Within the study, estimated O_3 concentrations at the residential address were derived from IDW, while the NO_2 concentrations were estimated using the same LUR model as $PM_{2.5}$. $PM_{2.5}$ was similarly correlated with both NO_2 and O_3 ($r = 0.55$). In a copollutant model with NO_2, the $PM_{2.5}$-lung cancer mortality association was attenuated and became null (HR = 0.99 [95% CI: 0.87, 1.11]), but remained relatively unchanged from the single-pollutant model result in a copollutant model with O_3 (HR = 1.10 [95% CI: 0.99, 1.22]). These results are consistent with those observed in Lipsett et al. (2011) in the CTS cohort. The authors reported that $PM_{2.5}$ was moderately to highly correlated with NO_X, CO, NO_2, and PM_{10} with correlations ranging from 0.52–0.91, but in copollutant models with O_3 the $PM_{2.5}$-lung cancer mortality association was relatively unchanged (HR = 1.04 [95% CI; 0.70, 1.53]) compared to the single-pollutant model result. The authors did not present results for copollutant models with the other pollutants examined.

Whereas the lung cancer mortality studies tended to report results for copollutant models with O_3, only Gharibvand et al. (2016) examined $PM_{2.5}$-lung cancer incidence associations in models with O_3. Within the AHSMOG-2 cohort, Gharibvand et al. (2016) observed that the $PM_{2.5}$-lung cancer incidence association was unchanged in copollutant models with O_3 (HR = 1.21 [95% CI: 1.02, 1.43]). Raaschou-Nielsen et al. (2013) within the ESCAPE study, also examined potential copollutant confounding of the $PM_{2.5}$-lung cancer incidence association, and did not find any evidence of confounding in models with NO_2 and $PM_{10-2.5}$ (quantitative results not presented).

Across the small number of studies that examined potential copollutant confounding of the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence, there is little evidence of copollutant confounding by O_3 with more limited information available to assess potential copollutant confounding for the other gaseous pollutants and particle size fractions. However, to date, studies have not systematically evaluated copollutant confounding across the gaseous pollutants.

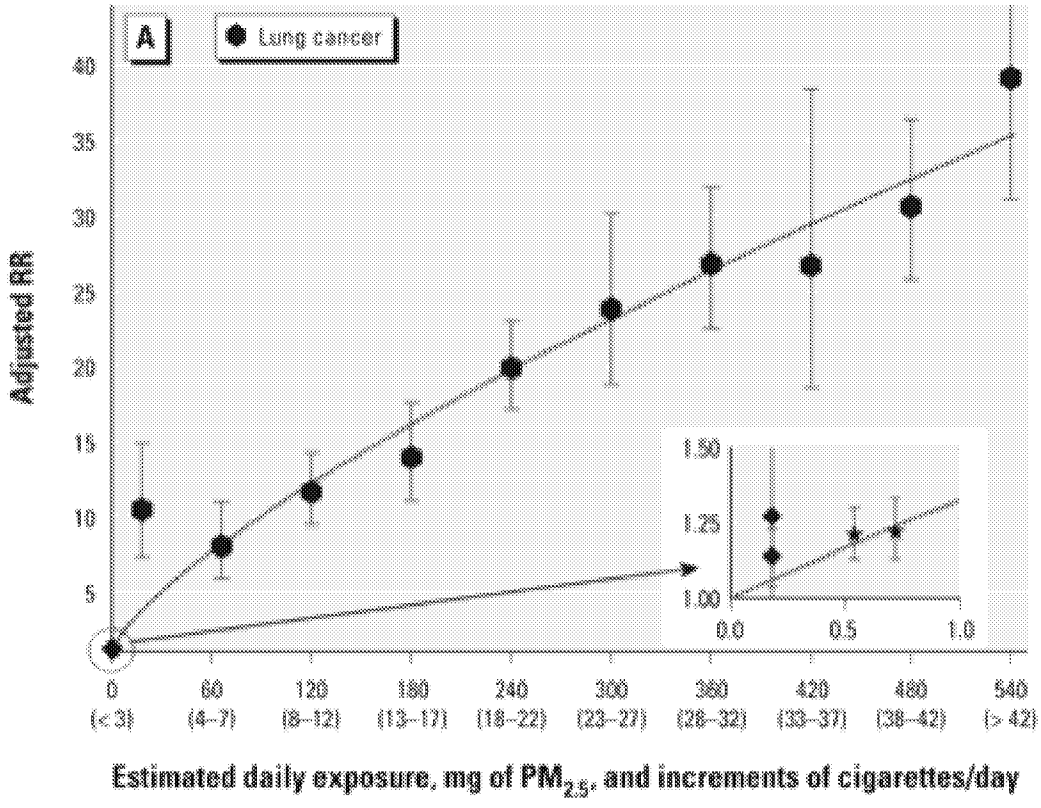
10.2.5.1.4 Concentration-Response (C-R) Relationship

Epidemiologic studies that examined the C-R relationship between long-term $PM_{2.5}$ exposure and mortality have generally found evidence of a log-linear, no threshold relationship (see Section 11.2.4). However, fewer studies have examined the C-R relationship for cause-specific mortality outcomes, including lung cancer. Recent cohort studies of both lung cancer mortality and incidence have examined both the shape of the C-R relationship along with whether there is evidence of a threshold, or level below which there is no effect.

Across the studies evaluated, a few provided information on the shape of the $PM_{2.5}$-lung cancer mortality (Lepeule et al., 2012) and lung cancer incidence (Puett et al., 2014; Raaschou-Nielsen et al., 2013) C-R relationship, but did not extensively discuss the results. Lepeule et al. (2012) in the HSC cohort along with Puett et al. (2014) in the NHS cohort and Raaschou-Nielsen et al. (2013) in the ESCAPE study reported no evidence for deviations from linearity in the shape of the C-R relationship when examining alternative models. Additionally, Cesaroni et al. (2013) in the RoLS cohort examined a 20% random sample of the full cohort to assess the C-R relationship, but the small sample size resulted in an underestimation of the $PM_{2.5}$-lung cancer mortality association and an inability to fully characterize the C-R relationship. Although these studies provide limited information on the shape of the $PM_{2.5}$-lung cancer mortality and incidence C-R relationship, studies by Pope et al. (2011) using the ACS cohort and Tomczak et al. (2016) using the CNBSS cohort conducted more extensive analyses.

Pope et al. (2011) examined lung cancer mortality, but to convey the public health burden associated with exposures to $PM_{2.5}$ of ambient origin compared the shape of the C-R relationship for lung cancer mortality across three different exposures: active smoking, SHS, and ambient $PM_{2.5}$ exposures. For this analysis the authors focused on only 6 years of follow-up due to the lack of smoking information after initial enrollment. Pope et al. (2011) calculated adjusted relative risks (RRs) for lung cancer

mortality due to smoking status using the ACS cohort data, and relied upon RRs from other cohort studies of lung cancer mortality due to long-term $PM_{2.5}$ exposure and SHS. Using the adjusted RRs and estimates of: average inhaled dose of $PM_{2.5}$ from active smoking; average daily dose of inhaled $PM_{2.5}$ based on the range of $PM_{2.5}$ concentrations from recent U.S.-based cohort studies and average inhalation rates; and dose from SHS exposure based on approximate $PM_{2.5}$ exposures and average inhalation rates, Pope et al. (2011) fit an integrated-exposure response function using a simple power function. This functional form was selected because it allows for nonlinearity in the C-R relationship (Pope et al., 2011). In a plot of the relative risks for lung cancer mortality for ambient $PM_{2.5}$ exposure, SHS, and active smoking in relation to the estimated daily dose of $PM_{2.5}$ from different increments of cigarettes per day in smokers compared to never smokers, the authors observed evidence of a nearly linear relationship (Figure 10-5). This relationship persisted when examining lung cancer mortality in both men and women, and when accounting for smoking duration.

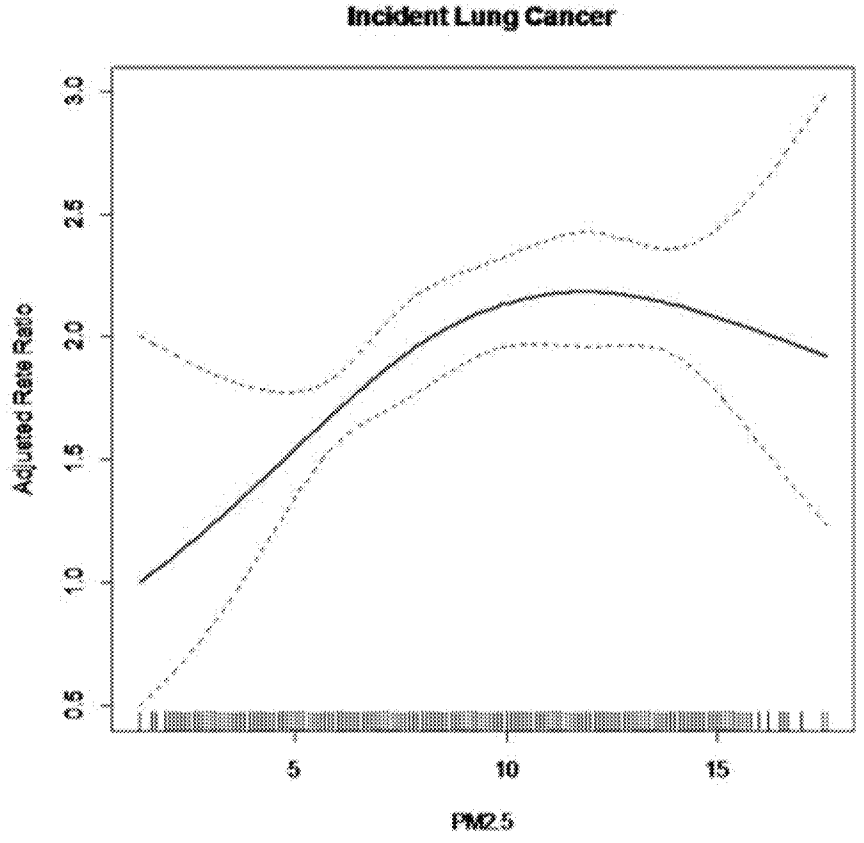


Note: Inset represents RR due to ambient $PM_{2.5}$ exposure and SHS. Diamonds = RR from studies of long-term $PM_{2.5}$ exposure and lung cancer mortality; stars = pooled RR estimates from studies of SHS and lung cancer mortality.

Source: Permission pending, Pope et al. (2011).

Figure 10-5 Adjusted relative risk (RR) for lung cancer mortality plotted over estimated daily dose of $PM_{2.5}$ (milligrams) and increments of cigarette smoking (cigarettes per day) compared to never smokers.

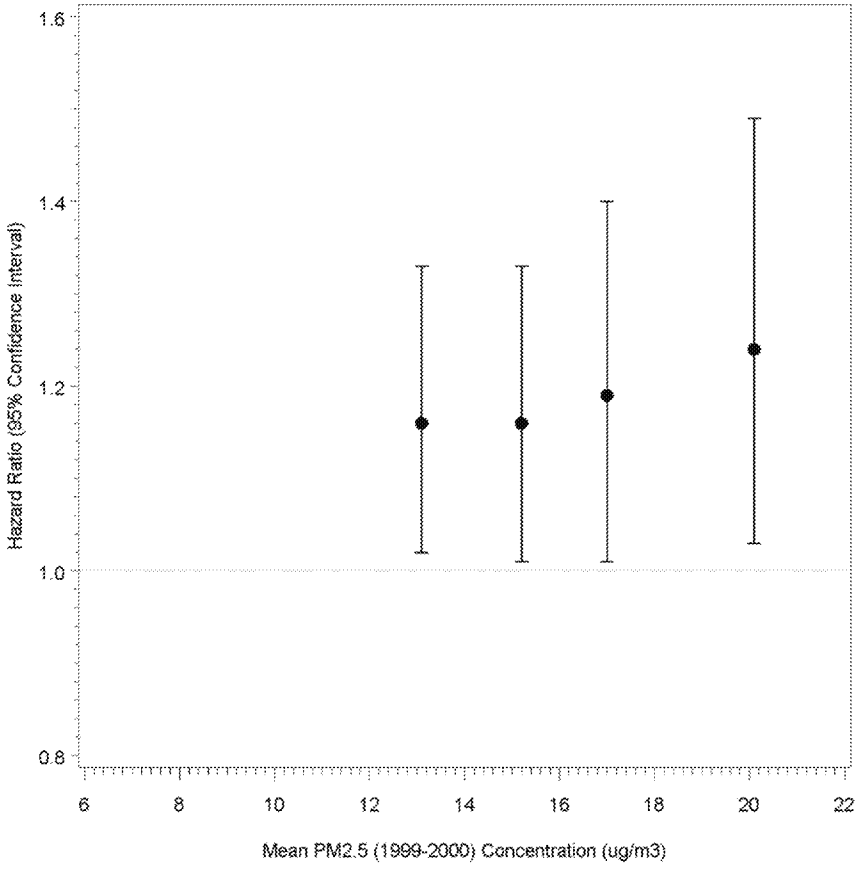
Tomczak et al. (2016) examined the shape of the C-R relationship for lung cancer incidence using the CNBSS. To examine whether there was evidence of nonlinearity in the C-R relationship, the authors considered a model with a natural cubic spline and 3 df. As depicted in Figure 10-6, Tomczak et al. (2016) observed evidence of nonlinearity in the $PM_{2.5}$-lung cancer incidence C-R relationship, which was depicted by a linear relationship up until approximately 12 $\mu g/m^3$ which then flattened out. The results of Tomczak et al. (2016) in this cohort of women differs from the examination of the C-R relationship in women by Pope et al. (2011) where the shape was found to be linear, which was consistent with the results of the full cohort. Although there is ambiguity in the shape of the C-R relationship above 12 $\mu g/m^3$ both Tomczak et al. (2016) and Pope et al. (2011) provide evidence of a linear C-R relationship in the range of $PM_{2.5}$ concentrations observed in the U.S.



Source: Permission pending, Tomczak et al. (2016).

Figure 10-6 Concentration-response (C-R) relationship between long-term $PM_{2.5}$ exposure and lung cancer incidence using a natural cubic spline and 3 degrees of freedom (df) in the Canadian National Breast Cancer Screening Survey (CNBCSS) cohort.

In addition to the studies that formally evaluated the C-R relationship, other studies used cut point analyses to examine whether there was evidence of a threshold or if the risk of lung cancer mortality or incidence varied across the range of $PM_{2.5}$ concentrations in each study. Turner et al. (2011) in the analysis of never smokers in the ACS-CPS II cohort examined the lung cancer mortality association across percentiles of the $PM_{2.5}$ distribution. When examining each percentile to the referent category, i.e., $PM_{2.5}$ concentrations less than 11.8 $\mu g/m^3$, the authors found relatively consistent associations with 95% confidence intervals increasing at higher concentrations, which is indicative of lower data density within those ranges of $PM_{2.5}$ concentrations (Figure 10-7).



Note: Cut-points represent the 25th (11.8 $\mu g/m^3$), 50th (14.3 $\mu g/m^3$), 75th (16 $\mu g/m^3$), and 90th (17.9 $\mu g/m^3$) percentiles.
Source: Permission pending, Turner et al. (2011).

Figure 10-7 Fully adjusted hazard ratios (95% confidence intervals) for lung cancer mortality in categorical analyses of mean $PM_{2.5}$ (1999–2000) concentrations in never smokers in the American Cancer Society-Cancer Prevention Study II (ACS-CPS II) cohort.

The results of Turner et al. (2011) in an analysis of lung cancer mortality, are consistent with those of Hystad et al. (2013) when examining lung cancer incidence in the NECSS cohort. In quintiles

that encompassed $PM_{2.5}$ concentrations less than those observed in Turner et al. (2011), ranging from less than 9.0 μg/m^3 for the referent category and above 14.7 μg/m^3 for the 5th quintile, the OR for long-term $PM_{2.5}$ exposure and lung cancer incidence ranged from 1.09−1.18, while the full cohort observed an OR = 1.14.

Instead of comparing $PM_{2.5}$-lung cancer incidence associations across a range of concentrations, Raaschou-Nielsen et al. (2013) in the ESCAPE study conducted a cut-point analysis to examine whether there was evidence of an association between long-term $PM_{2.5}$ exposure and lung cancer incidence below defined $PM_{2.5}$ concentrations. In the cut-point analysis, the authors excluded all participants with assigned $PM_{2.5}$ exposures that were above designated values (i.e., 10, 15, 20, and 25 μg/m^3). Across each of the cut-point values, Raaschou-Nielsen et al. (2013) reported consistent positive associations across each cut-point although confidence intervals were large due to the limited sample size for each cut-point value (HRs: 10 μg/m^3: 1.20 [95% CI: 0.55, 2.66]; 15 μg/m^3: 1.11 [95% CI: 0.85, 1.45]; 20 μg/m^3: 1.14 [95% CI: 0.90, 1.45]; 25 μg/m^3: 1.13 [95% CI: 0.90, 1.43]). The combination of results from cut-point analyses by Turner et al. (2011), Hystad et al. (2013), and Raaschou-Nielsen et al. (2013) collectively provide evidence indicating no threshold down to the lowest cut-point examined in each study (e.g., 9−11.8 μg/m^3).

Across the studies that examined long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence, evidence from analysis of the shape of the C-R relationship, cut point analyses, and threshold analyses all support a no-threshold, log-linear relationship across the range of $PM_{2.5}$ concentrations observed in the U.S. Although Tomczak et al. (2016) observed a potentially nonlinear C-R relationship, this plateauing of the $PM_{2.5}$ association occurred at concentrations higher than those observed in many areas of the U.S., and was not consistent with the results of Pope et al. (2011) when focusing on women in the ACS-CPS II cohort.

10.2.5.1.5 Summary

Since the completion of the 2009 PM ISA there has been a dramatic increase in the number of studies that examined the relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence using both previously examined cohorts as well as new cohorts. Collectively, these studies provide evidence of generally consistent, positive associations with both lung cancer mortality and incidence (Figure 10-3). These associations were observed across studies that adjusted for smoking status and exposure to SHS as well as those studies that had no direct measures of smoking status or used proxy measures to adjust for smoking.

In studies that conducted analyses on never smokers almost all of the studies, except a few conducted in Canada (Tomczak et al., 2016; Hystad et al., 2013) provided evidence of consistent positive associations. The positive associations for lung cancer in never smokers were confirmed by Turner et al. (2011) in a study of only never smokers in the ACS-CPS II cohort. The limited number of studies that

examined potential copollutant confounding reported that $PM_{2.5}$-lung cancer mortality and incidence associations remained relatively unchanged, specifically for O_3, with less evidence for other pollutants. Additionally, an examination of the C-R relationship and whether a threshold exists provided evidence that supports a no-threshold, log-linear relationship along the $PM_{2.5}$ concentrations observed in most locations within the U.S., specifically at concentrations representative of the lowest cut-point examined in studies, 9−11.8 $\mu g/m^3$, and where analyses of the C-R curve depict a widening of confidence intervals, ≈6 $\mu g/m^3$.

The collective body of evidence for lung cancer mortality and incidence detailed within this section, forms a substantial portion of the evidence included in recent meta-analyses of $PM_{2.5}$ and lung cancer risk, i.e., the meta-analyses did not delineate between lung cancer mortality and incidence in estimating the overall lung cancer risk (Chen et al., 2015; Yang et al., 2015; Cui et al., 2014; Hamra et al., 2014). Although the criteria for study inclusion varied across each of these meta-analyses they all reported evidence of a positive association between long-term $PM_{2.5}$ exposure and lung cancer risk (Figure 10-3). Specifically, the Hamra et al. (2014) meta-analysis, which formed a strong basis for the IARC conclusion on PM and lung cancer, included the majority of the studies evaluated within this section, the sole difference being this section did not focus on those studies that did not directly measure $PM_{2.5}$.

10.2.5.2 Other Cancers

The 2009 PM ISA concluded that there was no epidemiologic evidence supporting associations between long-term PM exposure in organs or systems other than the lung. However, the overall body of evidence was extremely limited. Since the completion of the 2009 PM ISA a number of studies have explored the relationship between long-term $PM_{2.5}$ exposure and other cancers including, but not limited to the breast and brain, with the majority focusing on cancer incidence. Of these studies, some had inherent limitations, such as an ecologic study design, and, therefore, are not the focus of this section and are available at: https://hero.epa.gov/hero/particulate-matter. Study characteristics including $PM_{2.5}$ concentrations, study population, and exposure assignment approach for the studies that examined other cancer sites are detailed in Table 10-6.

Table 10-6 Study specific details and $PM_{2.5}$ concentrations from recent that examined long-term $PM_{2.5}$ exposure and cancer in other organs or systems.

Study Years	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
Breast cancer					
†Hart et al. (2016)[a]	NHS II (U.S.)	$PM_{2.5}$: 1988–2007 Follow-up: 1993–2011	Cases: 3,416 Pop: 115,921	12.6[b]	Monthly spatiotemporal prediction model to geocoded residential address as detailed in Yanosky et al. (2014)
†Reding et al. (2015)[a]	Sister study (U.S.)	$PM_{2.5}$: 2006 Follow-up: 2003–2013	Cases: 1,749 Controls: 47,591	10.5	Regionalized universal kriging model, as detailed in Sampson et al. (2013), to baseline home address
†Andersen et al. (2016)	DNC (Denmark)	$PM_{2.5}$: 1990–2013 Follow-up: 1993 or 1999–2013	Cases: 1,145 Pop: 22,877	19.7	Danish air pollution dispersion modelling system to estimate concentrations at residential address as detailed in Jensen et al. (2001)
†Wong et al. (2016)[c,f]	(Hong Kong)	$PM_{2.5}$: 1998–2011 Follow-up: 1998–2011	Deaths: 111 Pop: 66,820	33.7	Combination of monitoring data, geospatial height information, and satellite data to estimate concentrations at geocoded residential address as detailed in Li et al. (2005) and Lai et al. (2010)
Brain cancer					
†Jørgensen et al. (2016)[a]	DNC (Denmark)	$PM_{2.5}$: 1990–2013 Follow-up: 1993 or 1999–2013	Cases: 121 Pop: 25,143	19.7	Danish air pollution dispersion modelling system to estimate concentrations at residential address as detailed in Jensen et al. (2001)
†McKean-Cowdin et al. (2009)[c]	ACS-CPS II (U.S.)	$PM_{2.5}$: 1979–1983/ 1999–2000 Follow-up: 1982–2000	Deaths: 1,284 Pop: 630,487	1979–1983: 21.1 1999–2000: 14.0 Average: 17.7	Average of all monitoring sites in each MSA

Table 10-6 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent that examined long-term $PM_{2.5}$ exposure and cancer in other organs or systems.

Study Years	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m^3	Exposure Assessment
Liver cancer					
†Pan et al. (2016)	REVEAL-HBV (Taiwan)	$PM_{2.5}$: 2006–2009 Follow-up: 1991–2009	Cases: 464 Population: 23,820	Main Island: 32.2 Penghu Islets: 24.2	Ambient monitoring data from 75 fixed-site monitors across the study locations and modified ordinary kriging as detailed in Liao et al. (2006). R^2 = 0.73
†Pedersen et al. (2017)[h]	ESCAPE (Europe)	$PM_{2.5}$: 2008–2011 Follow-up: 1985–2005	Cases: 256 Population: 156,211	DCH: 11.3 VHM and PP: 13.6	LUR model as detailed in Beelen et al. (2013) to home address
Leukemia					
†Winters et al. (2015)[a]	(Canada)	$PM_{2.5}$: 1975–1994 Follow-up: 1975–1994	Cases: 1,064 Controls: 5,039	11.4–11.7[e]	Combination of satellite and monitoring data at postal code of residential address as detailed in as detailed in Hystad et al. (2012)
†Badaloni et al. (2013)[a]	SETIL (Italy)	$PM_{2.5}$: 2005 Follow-up: 1998–2001	Cases: 620 Controls: 957	20.6–21.1[d]	National Integrated Model (MINNI), a dispersion model, to 4 km grid cell and estimated for each geocoded residence
†Heck et al. (2013)[a,g]	(California)	$PM_{2.5}$: 1998–2007 Follow-up: 1998–2007	Cases: 479[i] Controls: 26,159	17.2	Monitoring station within 5 miles from address at birth

Table 10-6 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent that examined long-term $PM_{2.5}$ exposure and cancer in other organs or systems.

Study Years	Cohort Location	Years Air Quality/Follow-up	Events/ Population	Mean Concentration µg/m³	Exposure Assessment
Multiple cancers					
†Heck et al. (2013)[a,g]	(California)	$PM_{2.5}$: 1998–2007 Follow-up: 1998–2007	Cases: 397 Controls: 26,159	17.2	Monitoring station within 5 miles from address at birth
†Lavigne et al. (2017)	(Canada)	$PM_{2.5}$: 1998–2012 Follow-up: 1998–2012	Cases: 2,044 Pop: 2,350,898	1st, 2nd, and 3rd trimester; entire pregnancy, 1st year: 9.6	Satellite-derived estimates to 1 km resolution then adjusted based on GWR to centroid of residential 6-digit postal code as detailed in van Donkelaar et al. (2015)
†Wong et al. (2016)[c,f]	(Hong Kong)	$PM_{2.5}$: 1998–2011 Follow-up: 1998–2011	Deaths: 1,408 Pop: 66,820	33.7	Combination of monitoring data, geospatial height information, and satellite data to estimate concentrations at geocoded residential address as detailed in Li et al. (2005) and Lai et al. (2010)

ACS-CPS = American Cancer Society-Cancer Prevention Study; DCH = Diet, Cancer and Health Study; DNC = Danish Nurse Cohort; GWR = geographically weighted regression; NHS II = Nurses' Health Study-II; REVEAL-HBV = Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus; SETIL = Study on the aetiology of malignancies in children; VHM and PP = Vorarlberg Health Monitoring and Promotion Program.

[a]Cancer incidence.

[b]Mean concentration obtained from Hart (2017a).

[c]Cancer mortality.

[d]Range of mean concentration across analyses conducted.

[e]Range of $PM_{2.5}$ concentrations across cases and controls.

[f]Wong et al. (2016) examined a range of cancers including all malignant, all digestive organs, lung, breast, female genital, male genital, urinary, and lymphohematopoietic.

[g]Heck et al. (2013) examined a number of types of childhood cancers including leukemia.

[h]Only 2 (DCH, Denmark [1993–1997] and VHM and PP, Austria [1985–2005]) of the 4 escape cohorts examined measured $PM_{2.5}$.

[i]397 cases of acute lymphoblastic leukemia and 82 cases of acute myeloid leukemia.

†Studies published since the 2009 PM ISA.

10.2.5.2.1 Breast Cancer

Hart et al. (2016) and Reding et al. (2015) examined the association between long-term $PM_{2.5}$ exposure and breast cancer incidence in two U.S.-based cohorts, NHS II and Sister Study cohorts, respectively. In both studies, the authors observed relatively little evidence of an association overall for

breast cancer incidence or by hormone receptor subtype. Hart et al. (2016) using a 48-month average of $PM_{2.5}$ concentrations reported a HR = 0.95 (95% CI: 0.89, 1.01) for breast cancer incidence, which is similar to the results observed using a cumulative exposure metric (quantitative results not reported). Reding et al. (2015) also reported relatively little evidence for an association with breast cancer incidence using annual average $PM_{2.5}$ concentrations, HR = 1.04 (95% CI: 0.94, 1.16). The results of both U.S.-based studies are consistent with Andersen et al. (2016) in Denmark within the Danish Nurse Cohort (DNC) study, which provided no evidence of an association between 3-year running mean of $PM_{2.5}$ concentrations and breast cancer incidence (HR = 1.00 [95% CI: 0.87, 1.14]). However, in a study conducted at much higher $PM_{2.5}$ concentrations (>30 $\mu g/m^3$) in Hong Kong, Wong et al. (2016) reported a positive association with breast cancer mortality (HR = 1.34 [95% CI: 1.12, 1.60]).

10.2.5.2.2 Brain Cancer

The examination of long-term $PM_{2.5}$ exposure and brain cancer consisted of studies focusing on both incidence (Jørgensen et al., 2016) and mortality (McKean-Cowdin et al., 2009). In the DNC study, which consisted of female nurses over the age of 44, Jørgensen et al. (2016) used a 3-year running average of $PM_{2.5}$ concentrations and found evidence of a weak positive association for brain tumor incidence (HR = 1.09 [95% CI: 0.72, 1.65]), but no evidence of an association when focusing on malignant brain tumors (HR = 0.97 [95% CI: 0.47, 2.05]). The lack of an association with brain cancer incidence was supported by the results of McKean-Cowdin et al. (2009), using the ACS-CPS II cohort, when examining brain cancer mortality. When using three different exposure metrics representing $PM_{2.5}$ concentrations from 1979–1983 (RR = 0.94 [95% CI: 0.87, 1.01]), 1999–2000 (RR = 0.98 [95% CI: 0.89, 1.09]), and the average of the two time periods (RR = 0.95 [95% CI: 0.86, 1.05]), the authors reported no evidence of an association with brain cancer mortality.

10.2.5.2.3 Liver Cancer

Recent studies conducted in Taiwan (Pan et al., 2016) and Europe (Pedersen et al., 2017) have examined the relationship between long-term $PM_{2.5}$ exposure and liver cancer incidence. Pan et al. (2016) within the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) cohort in Taiwan examined long-term $PM_{2.5}$ exposure based on 4-year average concentrations and liver cancer incidence on both the Main Islands and Penghu Islets. Additionally, the authors examined whether there was evidence of a direct or indirect effect of long-term $PM_{2.5}$ exposure on serum alanine transaminase (ALT) levels, which is a marker of chronic liver tissue inflammation, and subsequently liver cancer incidence. During the course of the study, new cases of liver cancer were identified during follow-up by pathological examination. Between the two locations, the distribution of $PM_{2.5}$ concentrations varied dramatically with an IQR of 0.73 μ/m^3 on the Penghu Islets and 13.1 $\mu g/m^3$ the Main Islands, therefore, results are not standardized to a 5 $\mu g/m^3$ increase, which as noted previously

is the convention for the rest of the epidemiologic study results for $PM_{2.5}$ presented within this section. Based on an IQR increase, Pan et al. (2016) reported a HR = 1.22 (95% CI: 1.02, 1.47) on the Penghu Islets and HR = 1.21 (95% CI: 0.95, 1.52) on the Main Islands. In the mediation analysis, there was evidence of an indirect effect of long-term $PM_{2.5}$ exposure on liver cancer incidence through elevated ALT levels, as well as some evidence of a potential direct effect. This initial evidence of a potential association between long-term $PM_{2.5}$ exposure and liver cancer is consistent with the results of Pedersen et al. (2017) in the ESCAPE study, which used a more rigorous exposure assignment method than Pan et al. (2016). Focusing on the two cohorts conducted in Denmark and Italy that reported $PM_{2.5}$ concentrations, the authors reported a positive association with new liver cancer cases diagnosed during follow-up (HR = 1.34 [95% CI: 0.76, 2.35]), but the 95% confidence intervals were large.

10.2.5.2.4 Leukemia

The association between long-term $PM_{2.5}$ exposure and incident leukemia was examined in cohorts consisting of children in Italy (Badaloni et al., 2013) and the U.S. (Heck et al., 2013), and adults in Canada (Winters et al., 2015). Badaloni et al. (2013) in the SETIL study (i.e., Study on the aetiology of lymphohematopoietic malignancies in children), examined incident leukemia in children ≤10 years of age in a case-control study. In quartile analyses using the entire cohort, as well as analyses limited to children between the ages of 0–4, and those children that did not change residence during the course of the study, the authors observed no evidence of an association between long-term $PM_{2.5}$ exposure and incident leukemia. Heck et al. (2013) examined incident childhood cancer (ages <6 years) from the California Cancer Registry. In a case-control study, the authors did not observe clear evidence of an association between $PM_{2.5}$ and acute lymphoblastic leukemia (OR = 1.06 [95% CI: 0.95, 1.18], n = 397) no evidence of an association with acute myeloid leukemia (OR = 0.90 [95% CI: 0.70, 1.16], n = 82). A similar result was observed by Winters et al. (2015) also using a case-control study design to examine incident leukemia in adults across Canadian provinces (except for Quebec and New Brunswick). The authors reported no evidence of an association between long-term $PM_{2.5}$ exposure and incident leukemia as well as chronic lymphocytic leukemia.

10.2.5.2.5 Multiple Cancers

Although most of the studies that examine long-term $PM_{2.5}$ exposure and cancer focused on specific cancer types, a few studies examined a number of different cancer types. Wong et al. (2016) in a study conducted in Hong Kong examined mortality attributed to a variety of cancers as detailed in Table 10-6. Within this study $PM_{2.5}$ concentrations were much higher (mean = 33.7 $\mu g/m^3$) compared to the other studies evaluated in this section. Across mortality outcomes attributed to cancer types, the authors observed strong positive associations (i.e., in terms of magnitude and precision) for all malignant, all

digestive organs, and female genital cancers with HRs ranging from 1.10 to 1.32. There was no evidence of an association for male genital, urinary, or lymphohematopoietic cancer mortality.

Whereas Wong et al. (2016) focused on cancer mortality, Heck et al. (2013) and Lavigne et al. (2017) examined incident childhood cancers in California and Ontario, Canada, respectively. Heck et al. (2013) in a case-control study, examined associations between $PM_{2.5}$ exposure during the entire pregnancy and childhood cancer (ages <6 years). There was not clear evidence of an association between $PM_{2.5}$ and cancer risk for any of the cancer sites except for retinoblastoma (OR = 1.33 [95% CI: 1.06, 1.67], n = 87). Lavigne et al. (2017) also examined multiple childhood cancers, but included cancer diagnoses up to age 14. In addition to examining exposures during the entire pregnancy, the authors also examined trimester specific exposures as well as those during the first year of life. Focusing on cancers with greater than 200 cases during the study period (i.e., acute lymphoblastic leukemia, astrocytoma, and Wilms tumor) the authors reported evidence of a number of positive associations across trimesters, the entire pregnancy, and the first year of life for each of these cancers, but 95% confidence intervals were large for all except astrocytoma (HR = 1.80 [95% CI: 1.09, 2.92] for the 1st trimester and HR = 1.68 [95% CI: 1.00, 2.89] for the entire pregnancy). These results are inconsistent with Heck et al. (2013), which also examined astrocytoma and found no evidence of an association with $PM_{2.5}$ exposure during the entire pregnancy.

10.2.5.2.6 Summary

Compared to the 2009 PM ISA, more recent studies have examined associations between long-term $PM_{2.5}$ exposure and cancer incidence and mortality beyond the respiratory system. Across the cancers examined, which includes breast cancer, brain cancer, liver cancer, and leukemia there is inconsistent evidence of an association with long-term $PM_{2.5}$ exposure. In addition to the cancers evaluated within this section, there are a few individual studies that examined ovarian cancer (Hung et al., 2012) and bladder cancer (Liu et al., 2009). Collectively, there are a small number of studies that examined other cancers and this evidence does not clearly depict an association between long-term $PM_{2.5}$ and cancer in other sites.

10.2.5.3 Cancer Survival

The majority of air pollution epidemiologic studies focusing on cancer tend to examine whether long-term exposures are associated with cancer incidence or mortality, as previously detailed within this section. Recently, studies have also examined whether exposure to air pollutants, such as $PM_{2.5}$, can have a detrimental impact on cancer survival. Study characteristics for the studies that examined cancer survival in response to long-term $PM_{2.5}$ exposures are detailed in Table 10-7.

Table 10-7 Study specific details and $PM_{2.5}$ concentrations from recent studies that examined cancer survival.

Study Location, Years, Data	Population/Cancer	Mean Concentration µg/m^3	Exposure Assessment	Results
†Xu et al. (2013) Los Angeles, CA; Honolulu, HI 1992–2008 SEER	58,586 respiratory cancer cases among whites LA: 56,193 Honolulu: 2,393	LA: 18.1 Honolulu: 4.3	Average of all monitors in the county where the case resided to calculate county-level monthly mean, each case assigned monthly mean concentration for each month after diagnosis.	Kaplan-Meier Survival Analysis: Higher mortality rate for respiratory cancer cases in areas with high $PM_{2.5}$ concentrations (LA) vs. low (Honolulu) Cox Proportional Hazards Model: Categorical analysis (LA only):[a] Overall mortality: HR = 1.07 (95% CI: 1.02, 1.13) Respiratory cancer mortality: HR = 1.08 (1.02, 1.14) Continuous variable analysis (per 5 µg/m^3): Overall mortality: HR = 1.57 (95% CI: 1.53, 1.61) Respiratory cancer mortality: HR = 1.49 (1.45, 1.53)

Table 10-7 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies that examined cancer survival.

Study Location, Years, Data	Population/Cancer	Mean Concentration µg/m^3	Exposure Assessment	Results
†Eckel et al. (2016) 1988–2009[b] California CCR	352,053 lung cancer cases	13.7	Monthly average concentrations interpolated to residential address using IDW of up to four closest monitors within 50 km radius; however, cases excluded if nearest monitor was >25 km away. Each case assigned monthly mean for each month after diagnosis.	Cox Proportional Hazards Model (per 5 µg/m^3): All-cause mortality: HR = 1.15 (95% CI: 1.15, 1.16) Lung cancer mortality: HR = 1.14 (95% CI: 1.13, 1.15)
†Hu et al. (2013) California 1999–2009 CA SEER	255,128 female breast cancer cases	—	Average of all monitors in the county where the case resided to calculate county-level monthly mean, each case assigned monthly mean concentration for each month after diagnosis. Cases excluded if any missing PM data during any month.	Kaplan-Meier Survival Analysis: Higher mortality rate for breast cancer cases living in counties with high $PM_{2.5}$ concentrations vs. low Cox Proportional Hazards Model: Breast cancer mortality: Categorical analysis:[d] 11.64–15.04 µg/m^3: 1.24 (95% CI: 0.79, 1.94) ≥15.04 µg/m^3: 1.76 (95% CI: 1.24, 2.49) Continuous analysis (per 5 µg/m^3): HR = 1.86 (95% CI: 1.12, 3.10)

Table 10-7 (Continued): Study specific details and $PM_{2.5}$ concentrations from recent studies that examined cancer survival.

Study Location, Years, Data	Population/Cancer	Mean Concentration µg/m^3	Exposure Assessment	Results
†Deng et al. (2017) California 2000–2009 CCR	22,221 HCC liver cancer patients	Total: 13.3 Local: 12.9 Regional: 13.3 Distant: 14.0	Same approach as described in Eckel et al. (2016) above.	Kaplan-Meier Survival Analysis: Median survival (years) was higher for all-cause mortality for liver cancer patients overall, and specifically for local and regional stage patients. Cox Proportional Hazards Model: Categorical Analysis:[e] Overall Results: 10–15 µg/m^3: 15–20 µg/m^3: 1.18 (95% CI: 1.12, 1.24) 20–25 µg/m^3: 1.46 (95% CI: 1.36, 1.57) 25–30 µg/m^3: 2.40 (95% CI: 2.14, 2.69) ≥30 µg/m^3: 4.61 (95% CI: 3.87, 5.50) Continuous Analysis (per 5 µg/m^3): 1.18 (95% CI: 1.16, 1.20)

CA SEER = California Surveillance Epidemiology and End Results cancer registry; CCR = California Cancer Registry; HHC = hepatocellular carcinoma; SEER = Surveillance Epidemiology and End Results cancer registry.

[a]Honolulu cases were the referent, for both categorical and continuous analysis results are for the fully adjusted model.

[b]For $PM_{2.5}$ analysis, only cases diagnosed in 1998 or later included.

[c]Mean $PM_{2.5}$ concentration not reported, but study conducted categorical analysis with $PM_{2.5}$ tertiles of <11.64 µg/m^3, 11.64–15.04 µg/m^3, and ≥15.04 µg/m^3.

[d]11.64 µg/m^3 was the referent, results are for the fully adjusted mode.

[e]<10 µg/m^3 was the referent.

†Studies published since the 2009 PM ISA.

Xu et al. (2013) and Eckel et al. (2016) examined cancer survival by focusing on both the influence of $PM_{2.5}$ concentrations on overall survival as well as the risk of death or cancer-related death in individuals with any respiratory cancer or lung cancer, respectively. Xu et al. (2013) focused on two areas representative of high (Los Angeles) and low (Honolulu) $PM_{2.5}$ concentrations, while Eckel et al. (2016) focused specifically on whether lung cancer cases resided in areas with higher and lower $PM_{2.5}$ concentrations. In Xu et al. (2013) and Eckel et al. (2016), cancer survival was found to decrease in areas with higher $PM_{2.5}$ concentrations, which was further supported by the categorical analysis conducted in Xu et al. (2013) where there was evidence of increased risk of mortality among people with cancer when comparing the higher polluted area (Los Angeles) with the lower polluted area (Honolulu). Additionally, in analyses in both studies where $PM_{2.5}$ was included as a continuous variable there was evidence of positive associations between long-term $PM_{2.5}$ exposure and overall mortality and respiratory/lung cancer mortality (Table 10-7).

Additional evidence indicating a potential relationship between cancer survival and long-term $PM_{2.5}$ concentrations was provided by studies conducted in California that examined breast cancer survival (Hu et al., 2013) and liver cancer survival (Deng et al., 2017). Hu et al. (2013) reported evidence of higher breast cancer mortality in cases living in counties with higher $PM_{2.5}$ concentrations as well as a high overall risk of breast cancer death. In the study of liver cancer survival, Deng et al. (2017) observed an overall increase in the risk of all-cause mortality as well as evidence that mortality risk increases in liver cancer patients as $PM_{2.5}$ concentrations increased (Table 10-7). Both of these studies provide initial evidence that although long-term $PM_{2.5}$ exposure has not been associated with breast cancer incidence, and only a few studies have examined liver cancer incidence (see Section 10.2.5.3), underlying cancer may contribute to increasing the risk of death after diagnosis.

In addition to examining overall cancer survival, Eckel et al. (2016), Hu et al. (2013), and Deng et al. (2017) examined whether the stage of cancer diagnosis modified survival. In each of these studies there was initial evidence, through categorical analyses, of a nonlinear relationship between $PM_{2.5}$ exposure and cancer survival, where patients with less advanced cancer at diagnosis (i.e., local or regional) had lower survival if they resided in locations with higher compared to lower $PM_{2.5}$ concentrations (Table 10-7). This pattern of associations was not observed in patients diagnosed with distant (i.e., late) stage cancer likely due to the advanced stage of cancer and overall lower survival rate. Collectively, these studies provide initial evidence that exposure to long-term $PM_{2.5}$ concentrations may contribute to reduced cancer survival. However, caution is warranted in the interpretation of the results from these studies because they are all conducted in one location, California.

10.2.6 Associations between $PM_{2.5}$ Sources and Components and Cancer

As characterized throughout this ISA, PM itself is a complex mixture consisting of numerous individual components derived from a variety of sources (see Chapter 2). It has been well characterized over the years that a number of these individual components are mutagenic, and carcinogenic (Claxton and Woodall, 2007; Claxton et al., 2004). The 2009 PM ISA noted that animal toxicological studies did not focus on specific PM size fractions, but instead emissions from various sources. The 2009 PM ISA concluded that ambient urban PM, emissions from wood smoke and coal combustion, and gasoline exhaust and DE are mutagenic, while PAHs are genotoxic. This conclusion is consistent with previous studies that demonstrated ambient PM and PM from specific combustion sources are mutagenic and genotoxic (U.S. EPA, 2009). Recent studies examined specific $PM_{2.5}$ components and in some cases related those components to specific sources to evaluate whether individual $PM_{2.5}$ components or sources are more closely related to lung cancer mortality and incidence, as well as DNA methylation, than $PM_{2.5}$ mass.

Thurston et al. (2013) in the National Particle Component and Toxicity (NPACT) study, which focused on the ACS-CPS II cohort, examined associations with individual $PM_{2.5}$ components and lung cancer mortality, and only observed evidence of positive associations with Se, a coal combustion tracer, and S. The authors used factor analysis and absolute principal component analysis (APCA) to identify source-related groupings and source categories, respectively. The results of the factor and source-apportionment analyses, which found positive associations with a Coal Combustion source, are consistent with the single-pollutant $PM_{2.5}$ component analyses. Thurston et al. (2013) did not observe evidence of clear associations with lung cancer mortality for any of the other source categories or tracer elements. (quantitative results not presented). The ESCAPE study also examined associations between long-term exposure to $PM_{2.5}$ components and lung cancer mortality. Raaschou-Nielsen et al. (2016) examined associations with eight $PM_{2.5}$ components (Cu, Fe, K, Ni, S, Si, V, and Zn) estimated using LUR methods. Positive associations were observed with all $PM_{2.5}$ components (with the exception of V), albeit with wide confidence intervals, with HR ranging from 1.02 to 1.34 for an IQR increase in $PM_{2.5}$ component concentrations.

Instead of focusing on traditional $PM_{2.5}$ components, Weichenthal et al. (2016) in the CanCHEC cohort examined the association between $PM_{2.5}$ oxidative burden (the product of mass concentration and oxidative potential) and lung cancer mortality. Regional time-weighted $PM_{2.5}$ (2012–2013) average oxidative potential was assessed according to the ability of filter extracts to deplete glutathione and ascorbate in synthetic respiratory tract lining fluid (percent depletion/μg). As detailed previously, there was a positive association with $PM_{2.5}$ mass that was found to be stronger in terms of magnitude and precision when using the glutathione-related $PM_{2.5}$ oxidative burden exposure metric (HR per IQR change in $PM_{2.5}$ and glutathione-related oxidative potential = 1.12 [95% CI: 1.05, 1.19]). There was no

association with ascorbate-related $PM_{2.5}$ oxidative burden (HR per IQR change in $PM_{2.5}$ and ascorbate-related oxidative potential = 0.97 [95% CI: 0.93, 1.01]).

In addition to studies that examined associations between $PM_{2.5}$ components and lung cancer mortality and incidence, a few studies examined whether specific $PM_{2.5}$ components are more strongly related to DNA methylation. Madrigano et al. (2011) within the Normative Aging Study discussed previously, also examined associations between individual $PM_{2.5}$ components and DNA methylation. In addition to $PM_{2.5}$ mass, the authors also observed associations for a reduction in methylation when examining BC and SO_4, particularly in LINE-1, but 95% confidence intervals were large. Additional studies conducted within the Beijing Truck Driver Air Pollution Study cohort detailed previously, also examined the influence of individual $PM_{2.5}$ components on DNA methylation. Hou et al. (2014) examined whether specific $PM_{2.5}$ components (i.e., Al, Ca, Fe, K, S, Si, Ti, and Zn) altered methylation of the same tandem repeats examined in Guo et al. (2014). The authors observed when examining associations for 10% increase in each component that there was evidence of an increase in SATα methylation for S in office workers and in NBL2 methylation for Si and Ca in truck drivers. However, Hou et al. (2014) did not examine components that comprised a larger percentage of $PM_{2.5}$ mass. For example, both Si and Ca represented less than 2 and 1% of the total $PM_{2.5}$ mass exposure for truck drivers and office workers, respectively. The authors reported no evidence of associations with other elemental components (Al, K, Ti, Fe, and Zn) or a difference in the methylation of the tandem repeat D4Z4. Sanchez-Guerra et al. (2015) also examined the Beijing Truck Driver Air Pollution Study cohort, but as detailed above focused on methylation of both 5mC and 5hmC. The authors did not report any evidence of an increase in 5hmC for the components examined in Hou et al. (2014) as well as BC.

Overall, the studies that examined associations between long-term exposure to $PM_{2.5}$ components and sources and lung cancer mortality are consistent with previous evaluations that have indicated that components and sources related to combustion activities are mutagenic and genotoxic and provide biological plausibility for PM-related lung cancer incidence and mortality (U.S. EPA, 2009). Additionally, initial evidence indicates that $PM_{2.5}$ oxidative potential may be an important metric to consider in the future. The limited number of studies that examined associations between exposure to $PM_{2.5}$ components and DNA methylation as well as the limited number of components examined, did not provide consistent evidence that any one component altered DNA methylation.

10.2.7 Summary and Causality Determination

It has been well characterized in toxicological studies that ambient air has mutagenic properties (Claxton et al., 2004) and that extracts of PM from ambient air have carcinogenic properties (Claxton and Woodall, 2007). However, at the completion of the 2009 PM ISA, little information was available from studies employing specific PM size fractions, such as $PM_{2.5}$, or inhalation exposure. The evidence indicating that PM was both a mutagen and carcinogen was supported by epidemiologic evidence of

primarily positive associations in studies of lung cancer mortality, with limited evidence for lung cancer incidence and other cancers. Since the 2009 PM ISA, a larger number of cohort studies using both traditional and more refined exposure assignment approaches provide evidence that primarily consists of positive associations between $PM_{2.5}$ exposure and both lung cancer mortality and lung cancer incidence, which is supported by subset analyses focusing on never smokers. In addition, $PM_{2.5}$ exhibits several key characteristics of carcinogens (Smith et al., 2016), as shown in toxicological studies demonstrating genotoxic effects, oxidative stress, electrophilicity, and epigenetic alterations, with supportive evidence provided by epidemiologic studies. Furthermore, $PM_{2.5}$ has been shown to act as a tumor promoter in a rodent model of urethane-initiated carcinogenesis. This biological plausibility, in combination with the epidemiologic evidence for $PM_{2.5}$ and lung cancer mortality and incidence, contributes to the conclusion of a likely to be causal relationship between long-term $PM_{2.5}$ exposure and cancer. This section describes the evaluation of evidence for cancer, with respect to the causality determination for long-term exposure to $PM_{2.5}$ using the framework described in Table II of the Preamble to the ISAs (U.S. EPA, 2015). The key evidence, as it relates to the causal framework, is summarized in Table 6-34.

Table 10-8 Summary of evidence for a likely to be causal relationship between long-term $PM_{2.5}$ exposure and cancer.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Consistent epidemiologic evidence from multiple, high quality studies at relevant $PM_{2.5}$ concentrations	Increases in lung cancer mortality and incidence in cohort studies conducted in the U.S., Canada, Europe, and Asia. Supported by subset analyses reporting positive associations in never smokers.	Section 10.2.5.1.1 Figure 10-3	Annual: U.S. and Canada: 6.3–23.6 Europe: 6.6–31.0 Asia: 33.7 Table 10-4
Limited epidemiologic evidence from copollutant models for an independent $PM_{2.5}$ association	Potential copollutant confounding for lung cancer mortality and incidence examined in a few studies with initial evidence that associations remained robust in models with O_3, with more limited information for other gaseous pollutants and particle size fractions.	Section 10.2.5.1.3	—
Epidemiologic evidence supports a log-linear, no-threshold concentration-response (C-R) relationship	Recent multicity studies conducted in the U.S., Canada, and Europe provide evidence of a log-linear, no-threshold C-R relationship for annual $PM_{2.5}$ concentrations observed within the U.S., but extensive systematic evaluations of alternatives to linearity have not been conducted.	Section 10.2.5.1.4	—

Table 10 8 (Continued): Summary of evidence for a likely to be causal relationship between long term $PM_{2.5}$ exposure and cancer.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Extensive evidence for biological plausibility	Experimental studies provide evidence for oxidative stress in human subjects while in vivo inhalation studies in rodents indicate oxidative DNA damage and methylation of a tumor suppressor gene promotor in the lung, upregulation of enzymes involved in biotransformation, and tumor promotion in a model of urethane-induced tumor initiation. Studies conducted in vitro show formation of DNA adducts, DNA damage, formation of micronuclei, oxidative stress, altered methylation of repetitive elements and miRNAs, increased telomerase activity, mutagenicity, and increased metastatic potential. Additionally, there is supporting epidemiologic evidence for micronuclei formation.	Liu et al. (2015) Soberanes et al. (2012) Yoshizaki et al. (2016) Cangerana Pereira et al. (2011) Section 10.2.1 Section 10.2.2 Section 10.2.3 Section 10.2.4 Section 10.2.5	238 µg/m^3 100–120 µg/m^3 594 µg/m^3 17.66 µg/m^3
Coherence of cancer-related effects across disciplines	Epidemiologic evidence that is coherent with experimental evidence for DNA adduct formation, DNA damage, cytogenetic effects, and altered DNA methylation	Li et al. (2014); Rossner et al. (2013b) Chu et al. (2015) Rossner et al. (2011) O'Callaghan-Gordo et al. (2015) Section 10.2.3	115.4 12.0–78.9 68.4–146.6 26.1–28.4 14.4

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated.

Experimental and epidemiologic studies provide evidence indicating the potential role of $PM_{2.5}$ exposure in genotoxicity through an examination of cancer-related biomarkers, such as mutagenicity, DNA damage, and cytogenetic endpoints. Decades of research has laid a foundation supporting the mutagenic potential of PM. It has been clearly demonstrated in the Ames *Salmonella*/mammalian-microsome mutagenicity assay that PM contains mutagenic agents (Section 10.2.2.1). Although mutagenicity does not necessarily equate to carcinogenicity, the ability of PM to elicit mutations provides support for observations of an association with lung cancer mortality and incidence in epidemiologic studies. Both in vitro and in vivo toxicological studies indicate the potential for $PM_{2.5}$ exposure to result in DNA damage (Section 10.2.2.2), which is supported by limited evidence from epidemiologic panel studies (Chu et al., 2015) and findings of oxidative stress in a controlled human exposure study (Liu et al., 2015). When examining cytogenetic effects, a limited number of epidemiologic

and toxicological studies provides coherence for micronuclei formation and chromosomal abnormalities (Section 10.2.2.3). Additionally, there was limited evidence for differential expression of genes that may be relevant to cancer pathogenesis. Across scientific disciplines, a broad array of biomarkers of genotoxicity were examined, which complicates the assessment of whether there was evidence for coherence of effects, but overall these studies provide some evidence of a relationship between $PM_{2.5}$ exposure and genotoxicity. Similarly, experimental and epidemiologic studies that examined epigenetic effects indicate changes in methylation, both hyper- and hypomethylation, globally as well as in some specific genomic sites, providing some support for $PM_{2.5}$ exposure contributing to genomic instability (Section 10.2.3). Toxicological evidence that the promoter region of a tumor suppressor gene, p16, was methylated in lung tissue as a result of inhalation exposure to $PM_{2.5}$ is consistent with one of the hallmarks of cancer (Hanahan and Weinberg, 2000); (Hanahan and Weinberg, 2011), i.e., the evading of growth suppressors (Section 10.2.3.1).

The experimental and epidemiologic evidence for genotoxicity and mutagenicity, as well as epigenetic effects, provides biological plausibility for a relationship between exposure to $PM_{2.5}$ and cancer development. In addition, $PM_{2.5}$ exposure enhanced tumor formation in an animal model of urethane-induced tumor initiation (Cangerana Pereira et al., 2011). This study supports a role for $PM_{2.5}$ exposure in tumor promotion, which is a measure of carcinogenic potential. Further substantiating the link between $PM_{2.5}$ exposure and cancer development are epidemiologic studies demonstrating primarily consistent positive associations between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence across studies using different exposure assignment methods (Section 10.2.5.1). The evidence of $PM_{2.5}$-related lung cancer mortality and incidence is further supported by a number of studies that examined associations by smoking status and reported generally positive associations in never smokers. Across studies, potential confounding by smoking status and exposure to SHS was adequately controlled through either direct measures of smoking status or by using proxy measures to adjust for smoking. Of those studies that did not report evidence of a positive association, only Lipsett et al. (2011) in the CTS cohort examined associations by smoking status for lung cancer mortality and also reported evidence of a positive, albeit imprecise, association in never smokers. A number of the studies focusing on lung cancer incidence examined associations by histological subtype, which allows for an assessment of adenocarcinoma, the only lung cancer subtype found in nonsmokers. Across studies that examined histological subtypes, there was some evidence of positive associations with adenocarcinomas, but associations were imprecise (i.e., wide confidence intervals) and often also observed for other subtypes.

A limited number of recent lung cancer mortality and incidence studies conducted analyses to assess potential copollutant confounding and reported that $PM_{2.5}$ associations were relatively unchanged in models with O_3. However, there was a more limited assessment of potential copollutant confounding by other gaseous pollutants and particle size fractions (Section 10.2.5.1.3). Recent assessments of the C-R relationship between long-term $PM_{2.5}$ exposure and lung cancer mortality and incidence provide evidence of a log-linear, no-threshold relationship, specifically at concentrations representative of the lowest cut-point examined in studies, 9–11.8 $\mu g/m^3$, and where analyses of the C-R curve depict a widening of

confidence intervals, ≈6 μg/m^3. However, in assessing the C-R relationship, epidemiologic studies have not conducted empirical evaluations of potential alternatives to linearity (Section 10.2.5.1.4).

In addition to lung cancer mortality and incidence, a number of recent studies examined cancers of other sites including breast cancer, brain cancer, liver cancer, and leukemia. Across the studies, the evidence does not clearly depict an association with other types of cancers (Section 10.2.5.2). However, emerging evidence examining cancer survival in people diagnosed with various stages of different types of cancers including respiratory cancer, lung cancer, breast cancer, and liver cancer indicate that long-term $PM_{2.5}$ exposure may contribute to reduced cancer survival, particularly in individuals with less advanced cancer diagnoses (Section 10.2.5.3).

Collectively, experimental and epidemiologic studies provide evidence for a relationship between $PM_{2.5}$ exposure and genotoxicity, epigenetic effects, and carcinogenic potential. Uncertainties exist due to the lack of consistency in specific cancer-related biomarkers associated with $PM_{2.5}$ exposure across both experimental and epidemiologic studies, however $PM_{2.5}$ exhibits several characteristics of carcinogens. This provides biological plausibility for $PM_{2.5}$ exposure contributing to cancer development. **Overall, the combination of this evidence is sufficient to conclude that a causal relationship is likely to exist between long-term $PM_{2.5}$ exposure and cancer**.

10.3 $PM_{10-2.5}$ Exposure and Cancer

The 2009 PM ISA concluded that the overall body of evidence was "inadequate to assess the relationship between long-term $PM_{10-2.5}$ exposures and cancer" (U.S. EPA, 2009).[77] This conclusion was based on the lack of epidemiologic studies that examined $PM_{10-2.5}$ exposure and cancer in both the 2004 PM AQCD and the 2009 PM ISA.

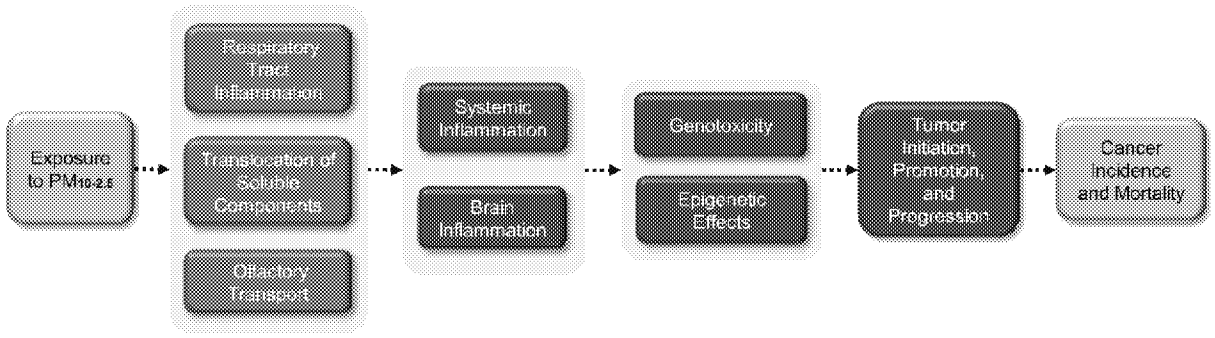
Consistent with the 2009 PM ISA, there remains a limited number of both experimental and epidemiologic studies that examined $PM_{10-2.5}$ exposure and whether it can lead to mutagenicity, genotoxicity, and carcinogenicity, as well as to cancer mortality. Although there is some evidence that $PM_{10-2.5}$ exposure can lead to changes in cancer-related biomarkers, there is a lack of epidemiologic evidence to support the continuum of effects to cancer incidence and mortality. The following sections evaluate studies published since completion of the 2009 PM ISA that focus on the mutagenicity, genotoxicity, and capability of long-term exposures to $PM_{10-2.5}$ to induce epigenetic changes all of which may contribute to cancer incidence and mortality.

[77] As detailed in the Preface, risk estimates are for a 5 μg/m^3 increase in annual $PM_{10-2.5}$ concentrations unless otherwise noted.

10.3.1 Biological Plausibility

This section describes biological pathways that potentially underlie the development of cancer resulting from exposure to $PM_{10-2.5}$. Figure 10-8 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to $PM_{10-2.5}$ may lead to the development of cancer contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 10.3.

Once $PM_{10-2.5}$ deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). $PM_{10-2.5}$ and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate ROS and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see Chapter 4). Immune system responses due to the presence of particles in the interstitial space may contribute to chronic health effects. Inflammatory mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (see Chapter 6). Although $PM_{10-2.5}$ is mostly insoluble, it may contain some soluble components such as endotoxin and metals. Soluble components of $PM_{10-2.5}$ may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of $PM_{10-2.5}$ may deposit on the olfactory epithelium. Soluble components of $PM_{10-2.5}$ may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4. The potential contribution of olfactory transport to brain inflammation or to upregulation of gene expression in the brain is discussed in Chapter 8.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 10-8 Potential biological pathways for the development of cancer following exposure to $PM_{10-2.5}$.

Evidence is accumulating that exposure to $PM_{10-2.5}$ may lead to carcinogenesis by a genotoxic pathway that may result in mutational events or chromosomal alterations. Carcinogenesis due to dysregulated growth may follow. Compared with $PM_{2.5}$, there is less evidence that $PM_{10-2.5}$ exhibits characteristics of carcinogens (Smith et al., 2016). However, exposure to $PM_{10-2.5}$ has been shown to result in genotoxic effects and to induce oxidative stress. Currently, epidemiologic evidence is limited to studies linking $PM_{10-2.5}$ exposure to lung cancer incidence. Evidence for these pathways and cancer-related biomarkers is described below.

Genotoxicity

Genotoxicity may occur as a result of DNA damage and subsequent introduction of mutations into the genome, and as a result of cytogenetic effects at the level of the chromosome. $PM_{10-2.5}$ exposure is associated with mutagenicity, DNA damage, and cytogenetic effects. Oxidative stress is one mechanisms involved in genotoxicity resulting from $PM_{2.5}$ exposure.

Mutations are considered biomarkers of early biological effect (Demetriou et al., 2012). Indirect evidence is provided by the Ames *Salmonella*/mammalian-microsome mutagenicity assay in one study. It can identify the presence of species that can result in mutations as the result of direct interactions with DNA as well as those that require metabolic activation to elicit genotoxicity. As the most widely accepted theory of cancer etiology is the accumulation of mutations in critical genes, the presence of mutagens within PM provides biological plausibility for observations made in epidemiological studies. While this assay has several technical limitations and is criticized due to its use of bacteria as a model species, four

decades of published results from this assay have clearly demonstrated the presence of mutagenic agents in PM collected from ambient air (U.S. EPA, 2009). A new study published since the 2009 PM ISA provides evidence to support mutagenicity resulting from $PM_{10-2.5}$ exposure (Kawanaka et al., 2008).

DNA damage is a biomarker of genotoxicity (Demarini, 2013). Evidence of DNA damage following $PM_{10-2.5}$ exposure was found using the comet assay in in vitro toxicological studies (Jalava et al., 2015; Wessels et al., 2010). The identification of oxidized DNA bases suggests a role for oxidative stress in the DNA lesions. These oxidized DNA nucleobases are considered a biomarker of exposure (Demetriou et al., 2012). Exposure to PM can result in oxidative stress either through the direct generation of ROS, or indirectly, through the induction of inflammation. Other in vitro studies demonstrated an increase in ROS production as a result of exposure to $PM_{10-2.5}$ (Section 10.3.2). A study in human subjects also found increased oxidized DNA bases in urine in association with $PM_{10-2.5}$ exposure (Liu et al., 2015). The presence of oxidative stress-mediated DNA lesions and adducts can lead to the introduction of fixed mutations into the genome after incorrect repair of the damaged base or replication past the base by low fidelity DNA polymerases. The potential for oxidative stress to result in mutagenesis is underscored by the DNA repair mechanisms that have evolved to protect the genome from mutagenesis caused by these lesions.

Cytogenetic effects, such as micronuclei formation and chromosomal aberrations, are biomarkers of genotoxicity (Demarini, 2013). Micronuclei are nuclei formed as a result of chromosomal damage, while chromosomal aberrations are modifications of the normal chromosome complement (Demetriou et al., 2012). Epidemiologic studies provide supportive evidence of micronuclei formation in association with $PM_{10-2.5}$ exposure (O'Callaghan-Gordo et al., 2015).

Summary of Biological Plausibility

As described here, there is one proposed pathway by which exposure to $PM_{10-2.5}$ may lead to the development of cancer. It involves genotoxicity, including DNA damage that may result in mutational events and cytogenetic effects that may result in effects at the level of the chromosome. While experimental studies in animals and humans contribute most of the evidence of upstream events, epidemiologic studies found associations between exposure to $PM_{10-2.5}$ and micronuclei formation. This proposed pathway provides biological plausibility for epidemiologic results of cancer incidence and mortality and will be used to inform a causality determination, which is discussed later in the chapter (Section 10.3.4).

10.3.2 Genotoxicity

In the 2009 PM ISA, there were a limited number of epidemiologic studies that examined molecular and cellular markers often associated with cancer, which includes both DNA damage and

cytogenetic effects. No studies specifically examined the effects of exposure to $PM_{10-2.5}$. Recent experimental and epidemiologic studies provide a limited body of evidence for genotoxicity due to $PM_{10-2.5}$ exposure.

10.3.2.1 Toxicological Evidence

Very few studies evaluating the genotoxicity and carcinogenicity of $PM_{10-2.5}$ have been published since the 2009 PM ISA. More common are reports detailing the effects in response to PM_{10}. However, as given the scope of the current ISA, only studies detailing the effects of $PM_{10-2.5}$ exposure are summarized here. While the Ames *Salmonella*/mammalian-microsome mutagenicity test was the most common method for analysis of genotoxicity in response to $PM_{2.5}$, the use of human cell culture and other in vitro assays were the primary method for the study of $PM_{10-2.5}$. No new studies published since the 2009 PM ISA that evaluated endpoints related to epigenetic changes in response to ambient air $PM_{10-2.5}$ exposure were identified.

Kawanaka et al. (2008) investigated the mutagenicity of roadside PM organic extracts from Saitama City, Japan. Using a cascade impactor, 12 fractions of varying aerodynamic diameters were collected including $PM_{10-2.5}$ (<0.12, 0.12–0.20, 0.20–0.30, 0.30–0.50, 0.70–1.2, 1.2–2.1, 2.1–3.5, 3.5–5.2, 5.2–7.8, 7.8–11, >11 μm). The authors used the *Salmonella* assay to determine the mutagenic activity of each fraction as well as GC/NCI/MS/MS and known quantities of select nitroaromatic compounds to determine the mass contribution of those compounds to the total PM collected and to estimate the contribution of each species to the total mutagenicity, respectively. Using this approach, it was reported that quantity of nitro-PAHs per unit mass in the ultrafine fraction was greater than that of $PM_{2.5}$ or $PM_{10-2.5}$. In addition, the authors determined that mutagenicity per unit mass of $PM_{10-2.5}$ was less than that of UFP (both TA98 and YG1024 S. Typhimurium strains) and that, of the six nitroaromatic compounds evaluated, the contribution to mutagenic activity calculated was greatest for 1,8-dinitropyrene in all three fractions of PM extracts evaluated. As a result of the variability of the *Salmonella* assay as well as incomplete details regarding the statistical analysis of the data collected, it is difficult to calculate definitive values for these contributions.

Jalava et al. (2015) used the alkaline comet assay to measure DNA damage after exposure to PM suspensions in mouse macrophages (RAW 264.7). They evaluated four size fractions including $PM_{10-2.5}$ collected at Nanjing University in China. The authors observed an increase in damage compared with controls ($p \leq 0.05$), however, the increase was observed only following exposure to the PM suspension of greatest concentration.

Wessels et al. (2010) also characterized the effect of exposure to $PM_{10-2.5}$ in cultured human cells. To represent and compare diverse PM mixture profiles, the authors collected PM from four locations including a rural location and three urban locations that varied in the extent to which vehicle traffic would contribute to the PM mixture sampled. Five size fractions were collected and that with the largest